GENETIC IMPROVEMENT OF *LEUCAENA* SPP. AND *ACACIA KOA* GRAY AS HIGH-VALUE HARDWOODS

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ABSTRACT

Studies on genetic improvement of *Leucaena* and *Acacia koa* Gray were undertaken in Hawaii from 1999-2003. There studies were described in two sections. The first section focused on research of *Leucaena* hybrids, which included vegetative propagation of *Leucaena* hybrids, hybrid yield trials, and chromosome doubling of diploid *Leucaena* species. Different hybrids rooted differently. Cuttings with more leaf presence had higher rooting frequency, better root qualities, and quicker root initiations. Leafless cuttings did not root under a mist system. One-node cuttings rooted as well as bi-nodal cuttings. Cuttings from younger (25-45 days old) regrown shoots rooted better than cuttings from older shoots (>45 days old). All hybrids rooted poorly in winter. Difficult-to-root hybrids rooted well after the treatments of etiolation. Three hybrid yield trials using clones of hybrids were carried out at three locations, Waimanalo of Oahu, and Hamakua and Kona of Hawaii Island. Seedless hybrid K1000 grew best at warm areas of Waimanalo and Kona, but grew poorly at cool area of Hamakua in terms of DBH, height, and wood volume. Two tetraploid hybrids, KX3 cl2 (*L. leucocephala* x *L. diversifolia*) and K156 x K376 (*L. diversifolia* x *L. pallida*) were among the fastest-growing hybrids at Waimanalo and Hamakua. The method (0.1% colchicine treatments on seedlings) to induce tetraploid *Leucaena* species was effective. Larger, thicker and darker leaflets, and larger flower heads of induced tetraploids were observed.

The second section focused on studies of koa, which included cytological study of koa, vegetative propagation, koa mortality and tolerance to wilt disease, and prediction of breeding values of koa. No variations in chromosome number were found among three

koa types and populations. The chromosome number of koa was identified as 2n=52 among all populations examined. Koa vegetative propagation was feasible at juvenile stage. Rooting ability of cuttings declined quickly with the increase of the age of cuttings. Cuttings of most families did not root at transitional and mature stages, and they did not respond to the treatments of growth regulators. Only cuttings of two families from Hawaii Island responded to auxins well with a moderate increase in rooting percentage at transitional and mature stages. Etiolation treatments appeared to have some effects on rooting at transitional stage. Koa survival rates in progeny trials declined steadily over the years. The mortality appeared to be mainly caused by koa wilt disease. The survival rates of koa families were analyzed and used to assess family tolerance to the disease. Great variation in survival rate was found among the families. Family selection based on survival rate was conducted. To rank the growth performance of koa families in progeny trials, breeding values of DBH at the age of 4 years were predicted in 4 progeny trials using BLP (Best Linear Prediction) method. Family ranking and selection based on predicted breeding values were conducted. Genetic gains of selection were calculated based on family selection.

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SECTION ONE

LEUCAENA RESEARCH

CHAPTER 1. LITERATURE REVIEW ON *LEUCAENA*

Leucaena, the genus of multi-purpose species, is widely used as livestock forage, fuelwood, reforestation material, green consumption, and green manure. Its uses have also been expanded to gum production, furniture and construction timber, pole wood, pulpwood, shade and support plants in agroforestry systems (NAS, 1979; Brewbaker, 1987a). The domestication of more species and development of interspecific hybrids has broadened its utilization (Brewbaker and Sorensson, 1990). In addition to its uses in the agricultural sector, its uses in industry have also burgeoned. Profitable generation of electricity using *L. leucocephala* biomass was recorded in the Philippines (Brewbaker, 1987a). *Leucaena* has also been used as pulpwood successfully and economically in Taiwan (Brewbaker, 1987a). It is also highly valued as an ecological remedy. Its capability of nitrogen fixation, rapid growth, and deep root system make it very useful in reforestation, bioremediation, and soil conservation. It suppresses invading grasses, and nurtures and stabilizes the soils (Oldfield, 1984).

Leucaena has undergone the same process of domestication and utilization as other crops have. *Leucaena leucocephala* was recognized as a good forage plant a long time ago. It reached the Philippines in the 1500s by Spanish sailors and spread around the tropics in the 19th century (Brewbaker and Hutton, 1979). However, the unimproved species had not been used widely and popularly until selected UH " Hawaiian Giant" varieties were released to the world in the 1960s. Since then, more intense breeding and improvement work has been conducted, notably in Hawaii, Australia, the Philippines, and the UK. Among the subjects of this research are breeding, collection and evaluation of germplasm, selection of varieties, hybridization, and studies of nutrition, and resistance to

insects, diseases, cold and acid environments. *Leucaena* is widely spread in the tropics, and it covers around 2 to 5 million hectares of land for various uses (Brewbaker and Sorensson, 1990). Three institutions are well known for their outstanding contributions to *Leucaena* research; they are the University of Hawaii, the Oxford Forest Institute, and the Commonwealth Scientific and Industrial Research Organization of Australia.

Taxonomy and cytology of genus *Leucaena*

Taxonomy

There are 22 species recognized by Hughes (1998), who listed an additional 9 subspecies and varieties in the genus *Leucaena*. The taxonomy of the genus *Leucaena* has been confusing since its establishment due to high intraspecific variations, and the difficulty of collecting adequate herbarium materials. The number of species identified has ranged from about 10 to more than 50. There is much debate about the delimitation of species and the classification of species and subspecies, often due to the paucity of excellent herbarium specimens. Historically, there are two trends in the taxonomy of *Leucaena*. One scholarly trend tend to exaggerate intraspecific variations and to inflate the number of species. Britton and Rose are among such scholars. They once brought the species number to 39, many of which are regarded as unreasonable and void today (Brewbaker, 1987b; Hughes, 1998). In contrast, persons on the other hand tend to be more conservative, and downplay the interspecific variations. Brewbaker and his colleagues are more skeptical of the proliferation of species number. In their initial classification, only 12 species were recognized on the basis of growth in research trials (Brewbaker, 1987b). Later, Sorensson (1993) described 16 species and 7 subspecies.

After careful inspection of living plants in the arboretum and wild, as well as herbarium of specimens, Hughes (1998) presented an updated classification including the 22 species in Table 1.1.

Research on species relationships in *Leucaena* involves cytological, morphological, and molecular data. Based on cytological and morphological data, Pan (1985) first proposed a phylogenetic scheme of the genus, where he placed *L. trichandra* at the base of the relational tree. All other taxa were derived from it. Harris et al. (1994) analyzed the variation of chloroplast DNA and used such information to construct a phylogenetic tree of the species. Due to maternal inheritance of chloroplast, such a relational tree is of limited value in the phylogeny of the genus, but is useful to reveal the possible origins of tetraploid species. The new scheme of species relationship derived from a cladistic analysis of 29 morphological characters provides evidence of the relationships within the genus. Based on this scheme, there are four species groups including esculenta, lanceolata, shannonii, and a group of other species, as proposed by Hughes (1998).

Cytology

There are two ploidy levels (2x and 4x) and two basic chromosome numbers (x=26 and x=28) in *Leucaena* species. Four chromosome numbers are found in the genus (2n=2x=52, 2n=2x=56, 2n=4x=104, and 2n=4x=112). While most species in the genus are diploid, 4 species are tetraploid and include the most economically important species like *L. leucocephala*, *L. pallida*, and *L. diversifolia*. The chromosome numbers of all 22 species have been determined (Table 1.1).

Table 1.1. Classification and chromosome numbers of 22 species of the genus *Leucaena*.

Species	Intraspecific taxa	2n	Author
L. collinsii Britton & Rose	subsp.*collinsii*	52	Pan and Brewbaker (1988); Sorensson (1989)
	subsp. *zacapana*	56	Schifino-Wittmann et al. (1999)
L. confertiflora S. Zarate	var. *confertiflora*	104	Schifino-Wittmann et al. (1999)
	var. *adenotheloidea*	112	Pan and Brewbaker (1988); Sorensson and Brewbaker (1994); Palomino et al. (1992; 1995); Schifino-Wittmann et al. (1999)
L. cuspidata Standley		52	Schifino-Wittmann et al. (1999)
L. diversifolia (Schltdl.) Benth		104	Pan and Brewbaker (1988); Sorensson (1989)
L. esculenta (Sesse & Moc. ex DC.) Benth		52	Hutton (1981); Pan and Brewbaker (1988); Sorensson (1989); Palomino et al. (1995); Schifino-Wittmann et al. (1999)
L. greggii S. Watson		56	Sorensson (1989)
L. involucrata S. Zarate		112	Schifino-Wittmann et al. (1999)
L. lanceolata S. Watson	var. *lanceolata* var. *sousae*	52	Gonzalez et al. (1967); Pan and Brewbaker (1988); Sorensson (1989)
L. lempirana C.E. Hughes		52 and 56	Schifino-Wittmann et al. (1999)
L. leucocephala (Lam.) de Wit	subsp. *leucocephala* subsp. *glabrata* subsp. *ixtahuacana*	104	Gonzalez et al. (1967); Pan and Brewbaker (1988); Sorensson (1989)
L. macrophylla Benth.	subsp. *macrophylla* subsp. *istmensis*	52 and 56	Sorensson (1989); Schifino-Wittmann et al. (1999)
L. magnifica (C.E. Hughes) C.E. Hughes		52	Sorensson (1989); Schifino-Wittmann et al. (1999)
L. matudae (S. Zarate) C.E Hughes		56	Sorensson (1989); Schifino-Wittmann et al. (1999)
L. multicapitula Schery		52	Sorensson (1989); Schifino-Wittmann et al. (1999)
L. pallida Britton & Rose		104 and 112	Pan and Brewbaker (1988); Sorensson (1989); Palomino et al. (1992); Schifino-Wittmann et al. (1999)
L. pueblana Britton & Rose		52	Schifino-Wittmann et al. (1999)
L. pulverulenta (Schltdl.) Benth.		56	Gonzalez (1967); Hutton (1981); Pan and Brewbaker (1988); Sorensson (1989)
L. retusa Benth.		56	Pan and Brewbaker (1988); Sorensson (1989)
L. salvadorensis Standley ex Britton & Rose		56	Sorensson (1989); Schifino-Wittmann et al. (1999)
L. shannonii J.D.Smith		52	Hutton (1981); Pan and Brewbaker (1988); Sorensson (1989)

Table 1.1. (Continued) Classification and chromosome numbers of 22 species of the genus *Leucaena*.

Species	Intraspecific taxa	2n	Author
L. trichandra (Zucc.) Urban		52 and 104	Hutton (1981); Pan and Brewbaker (1988); Sorensson (1989); Palomino et al. (1992); Schifino-Wittmann et al. (1999)
L. trichodes (Jacq.) Benth.		52	Gonzalez (1967); Hutton (1981); Pan and Brewbaker (1988)

The evolutionary pattern of chromosome numbers in *Leucaena* is unclear. Cytological and genetic evidence indicates that most tetraploid species are allotetraploids, and that tetraploid species *L. diversifolia* may be an autotetraploid derived from chromosome doubling of diploid species (Pan, 1985). Hughes (1998) also suggested that the tetraploid species are allotetraploids derived from hybridization between two diploid species.

Cytological studies of meiosis of 7 interspecific hybrids of 6 species reveal that chromosomes of different species are highly homologous to each other (Pan, 1985). This indicates the relationship of genomes of *Leucaena* species is very close, which also explains the high fertility of most interspecific hybrids.

Extra chromosomes are frequently found in a wide range of accessions of *L. trichandra* and *L. diversifolia* (Pan, 1985). The origin and role of the extra chromosomes is unknown.

Sun (1996) conducted a study on nuclear DNA contents of 17 *Leucaena* species. The 2C nuclear DNA contents of the species ranged from 1.33 to 1.74 pg for diploids and from 2.67 to 3.09 pg for tetraploids. The DNA content corresponded to approximately 650 megabase pairs for diploids, and 1,500 megabase pairs for tetraploids. DNA content and ploidy levels were highly correlated. Variations in DNA content were found among both diploids and tetraploids, even among species with the same chromosome number. The result of grouping the species based on DNA content was similar to that based on leaflet size of the species, and species with large leaflets usually have high DNA contents. The result of the study provides supportive evidence for species grouping and the possible origin of two tetraploid species *L. diverifolia* and *L. pallida,* which are

proposed to be derived from *L. trichandra*, and *L. trichandra* and *L. esculenta* respectively. Data on DNA content might be useful for future molecular studies of the genus (Sun, 1996).

Commercial and lesser-known species

L. leucocephala as a forage plant

Leucaena used to be referred to solely as *L. leucocephala* before other species of the genus were introduced into breeding programs. Although *L. leucocephala* was recognized as a good forage for animals as early as 400 years ago, its wide spread did not occur until its arboreal form, named "Hawaiian Giant" was found and released in the 1960s (Brewbaker, 1980, 1987a). Currently, *L. leucocephala* is the most commercialized species in the genus.

Three types of *L. leucocephala* have been identified in terms of their tree form, and leaflet and seed size. They are "Salvador", "Peru", and "Common", corresponding to giant, intermediate, and shrubby forms. Hughes (1998) further classified the species into three subspecies. The subspecies *glabrata* includes the "Salvador" and "Peru" types, whereas the subspecies *leucocephala* is also known as the "Common" type. Hughes (1998) identified a third subspecies known as "*ixtahuacana*".

Wide utilization of *L. leucocephala* is attributed to its high quality foliage, high adaptability to various environments, rapid growth, and high forage yield. Its yield ranges from 3 to 30 t dry matter/ha/year (Shelton and Brewbaker, 1994). The nutritional value of *L. leucocephala* leaf is fairly high compared to other forage species and other *Leucaena* species. Its forage features high protein content (27 to 34% of crude protein),

low fiber content, and balanced mineral elements, as well as high palatability (Norton et al., 1995). Mimosine, a toxic, non-protein amino acid, imposes adverse effects on animals, but it is destroyed in animal rumens by the bacterium *Synergistes jonesii* (Shelton and Jones, 1995).

Leucaena leucocephala to some extent is very adaptive to various tropical environments, especially to drought. However, low pH (<5.0), low Ca and high Al of soil, water-logging, and low temperatures do limit its growth (Brewbaker, 1987a; Shelton and Jones, 1995). For this reason, the species has not been spread to areas with acid soils and low temperatures. Introduction of genes tolerant to acid soils and to low temperatures from other species may be a solution to these problems. Partial success on these issues has been achieved in interspecific hybrids such as KX3 and KX2 (Brewbaker and Sorensson, 1990). The pysllid (*Heteropsylla cubana* Crawford) infection since the 1980s has been a devastating threat to *Leucaena*, which has not yet been fully resolved despite the enormous efforts that have been made.

Before the 1990s, genetic improvement of *Leucaena* was mainly focused on *L. leucocephala*. Numerous trials had been carried out to evaluate adaptation, psyllid resistance, and yield of *L. leucocephala* collections in the tropics from South Asia to Africa. Detailed information can be found in LEUCAENA RESEARCH REPORTS, VOL. 1-12. These trials provide supportive information for *Leucaena* planting in a specific area.

Leucaena species other than L. leucocephala

The genetic base of current *L. leucocephala* grown around the world is very narrow (Brewbaker, 1985). A large population probably originates from a single seed (Sun, 1992, Brewbaker, personal comm.). Hughes (1998) described *L. leucocephala* as an extreme example of narrow genetic base in tropical tree plantings. It has been long proposed to increase the genetic diversity of *L. leucocephala* to enhance resistance to insects and to increase adaptations to diverse environments such as acid soils, highlands, water-logging areas, where *L. leuceocephala* does not grow well (Brewbaker and Sorensson, 1990; Brewbaker and Sorensson, 1987; Shelton and Jones, 1995). Expanding the use of *Leucaena* (for example, as wood production and fuelwood) also calls for more attention to those underexploited species in the genus (Brewbaker and Sorensson, 1994). Early work on lesser-known species for potential uses started in 1980s in Hawaii (Brewbaker, personal comm.). Recent work has been summarized in LEUCAENA—ADAPTATION, QUALITY AND FARMING SYSTEM, ACIAR PROCEEDINGS NO. 86.

There are two approaches to incorporate lesser-known species into *Leucaena* plantations: by direct use of lesser-known species or hybridization between *L. leucocephala* and lesser-known species. Recently, a series of projects have been carried out to test the performance of lesser-known *Leucaena* species and hybrids as forage and wood sources throughout Southeast Asia, New Guinea, Australia, and Kenya. One such project assessed 25 accessions of *Leucaena* in 61 "environments " at 19 sites (Mullen, et al., 1998a, 1998b). The effects of accession and environment, and the interaction of genetic and environment effects (G x E) were analyzed to identify broad and specific

adaptations to environmental limitations. A UH interspecific hybrid, KX2 (*L. pallida* K376 x *L. leucocephala* K8), showed comparatively high yields and broad adaptations. *Leucaena trichandra* OFI 53/88 displayed specific adaptations to low temperatures in highlands. However, no accessions were found to adapt to acid, infertile soils or to low rainfall. In any specific site, some lesser-known species other than *L. leuceocephala* showed high forage yields and high adaptabilities to adverse environments.

Another project was carried out to test a foundation collection with more than 120 accessions in two locations, Australia and the Philippines (Mullen and Shelton, 1998; Gabunada and Stur, 1998). Agronomic potential and adaptation to adverse environments such as low temperatures and psyllid infection were evaluated. Again, KX2 showed the highest forage productivity at both sites. Results indicate significant potential of lesser-known species as effective forages with respect to their high yields and adaptations to adverse environments.

An important way to incorporate lesser-known species into *Leucaena* improvement is by artificial hybridization of *Leucaena* species. Interspecific hybrids of *Leucaena* have greatly expanded the uses of *Leucaena* as a multi-purpose tree, since the hybrids are more adaptive to environments and more productive (Brewbaker and Sorensson, 1990).

Attention has been paid to exploiting the tetraploid version of diploid species in order to use lesser-known species more effectively. Diploid species are of high value in *Leucaena* breeding programs. However, due to the difference of ploidy levels, crosses between tetraploid *L. leucocephala* and diploid species are less successful than those between *L. leucocephala* and tetraploid species (Sorensson, 1993). Colchicine-induced

tetraploids or unreduced gametes of diploids may facilitate gene transfers from diploid species to *L. leucocephala* (Brewbaker and Sorensson, 1990).

The frequencies of unreduced gametes in *Leucaena* species are generally low (< 1.0%), with the exception of some accessions of *L. pulverulenta* and *L. trichandra*, which produce up to 7.5 and 12% unreduced pollens respectively (Schifmo-Wittmann and Simioni, 1999; Sorensson and Brewbaker, 1987). Using unreduced gametes of certain accessions in breeding programs is promising.

Tetraploid versions of diploid *Leucaena* species have also been proposed to broaden crosses among species. In addition to more interspecific tetraploid hybrids, more triploids could be produced either from interspecific hybridization or intraspecific hybridization between two ploidy versions of the species.

Hybridization and genetic improvement of *Leucaena*

Intensive hybridization in genus *Leucaena* did not take place until late the 1980s, although the utilization of the sporadic interspecific hybrid of *L. pulverulenta* and *L. leucocephala* can be traced back as early as the 1940s in Indonesia. This natural hybrid is a partially sterile triploid of fast growth and cold tolerance, and is widely used for shading plants in coffee and tea plantations through grafting (Lammers, 1940; Brewbaker, 1988). Sporadic hybrids have also been found in other *Leucaena* species. For example, a vigorous and sterile hybrid from natural crosses between *L. esculenta* and *L. leucocephala* was found in Colombia (Hutton and Eddie, 1982).

Early artificial hybridization had been focused on the improvement of forage quality, yield, and adaptability of *L. leucocephala*. Gonzalez et al. (1967) crossed *L.*

leucocephala with *L. pulverulenta*, *L. trichodes*, *L. lanceolata*, and *L. esculenta* in Hawaii. Hybrids with intermediate mimosine contents were obtained from the cross between high mimosine content species *L. leucocephala* and low mimosine *L. pulverulenta*. Bray (1984) reported that *L. pulvrulenta* x *L. leucocephala* hybrids produced 50% more edible dry matter than the control--*L. leucocephala* K500, and up to 100% more wood over a two-year period. However, this hybrid is highly psyllid susceptible, which limits its uses (Brewbaker, personal comm.). Hutton (1988) reported hybridizations between *L. leucocephala* and *L. shannonii*, and between *L. leucocephala* and *L. esculenta*. The hybrids were more resistant to high Al and more tolerant to low Ca than *L. leucocephala*. Apparently, the resistance was inherited from *L. shannonii* and *L. esculenta*. Wong et al. (1998) focused on the problem of acid soils in Malaysia through hybridizing *L. leucocephala* with acid and psyllid tolerant species *L. diversifolia*. Two hybrids selected in F2 and F3 were superior to the best *L. leucocephala* varieties in edible dry matter, vigor, and psyllid tolerance.

The hybridization of *Leucaena* species has also been used to solve systematic problems of the genus in addition to genetic improvement. Pan (1985) made a dozen interspecific crosses to solve the puzzle of the taxonomy of some species and to reveal possible origins of tetraploid species. However, no hybrids validated his proposal of possible origins of tetraploids such as *L. leucocephala*, *L. diversifolia,* and *L. pallida* (Pan, 1985).

Sorensson (1987, 1993) reported on extensive hybridizations of *Leucaena*. Sixteen taxa were intercrossed in 97% of all 240 possible combinations. Viable seeds were produced in 61% of the combinations, and 99 hybrids were grown from 141

combinations producing viable seeds. This is the most intensive hybridization work in the genus, and much knowledge of the inheritance of botanical and agronomic characters, and the systematics of genus has been learned from those crosses. Several hybrids from the hybridization are promising for direct utilization including fodder and gum production, furniture, construction and polewood material, fuelwood, pulpwood, roundwood, charcoal, parquet and craftwood (Brewbaker and Sorensson, 1990).

Interspecific hybrids of *Leucaena* showed a good combination of characters of parents as well as bonus characters such as seedlessness. Hybrids from crosses among three tetraploid species, *L. leucocephala*, *L. diversifolia*, and *L. pallida* are notable for their excellent performance in forage and wood yield, resistance to insects, and tolerance to adverse environments such as low temperatures and acid soils (Brewbaker and Sorensson, 1990). One hybrid, known as KX2 (*L. leucocephala* x *L. pallida*) has especially raised researchers' interest for its high resistance to psyllids (Austin, 1995), and high forage yields across various environments (Austin, 1995; Sun, 1996; Mullen et al., 1998a). Another hybrid, K1000 (*L. leucocephala* x *L. esculenta*), is seedless, grows fast, and has good wood qualities. It is suitable for wood production and reforestations although its bad forage quality makes it unqualified for animal feeding (Brewbaker, personal comm.). Hybrids between diploids, such as *L. trichandra*, *L. esculenta, L. pulverulenta*, and tetraploids, *L.* diversifolia, *L. leucocephala*, are also promising (Brewbaker and Sorensson, 1990).

Genetic improvement of *Leucaena* has been focused on germplasm collection and evaluation, as well as intraspecific and interspecific heterosis, and recurrent selection. Early breeding work has been focused mainly on germplasm collection and evaluation.

Most of work had been summarized in LEUCAENA RESEARCH REPORTS, VOL. 1-12. Crosses among *L. leucocephala* collections reveal that there is a mild intraspecific heterosis of forage yield and wood biomass (Sun, 1996). This result is in contradiction with that of Gupta (1986), which shows no heterosis of forage and fuelwood yield in *L. leucocephala*.

Interspecific heterosis of KX2 (*L. pallida* x *L. leucocephala*) is very high, averaging 48% for forage yield and 85% for wood biomass. It is also tolerant to moderate cold temperatures and highly resistant to psyllids (Sun, 1996; Brewbaker, personal comm.). Recurrent selection has been conducted to capture intraspecific and interspecific heterosis (Sun, 1996). Recurrent lines are selected lines from advanced generations of intraspecific or interspecific hybrids. Compared to F1 hybrids, which rely on tedious hybrid seed production or costly vegetative propagation of F1 hybrids, recurrent lines are more economical on seed production since they are open-pollinated, but still maintain major heterosis in polyploids (Sun, 1996; Austin et al., 1998). Recurrent selection on hybrid K376 (*L. pallida*) x K8 (*L. leucocephala*) is effective with 98% of genetic gain after two cycles (Sun, 1996), and recurrent F2 lines of KX2 have been released to growers (Austin et al., 1998).

Leucaena **used in wood production**

Wood quality of L. leucocephala

Leucaena wood has a good reputation and is widely used for fuelwood, poles, furniture, and pulp. However, research on wood properties of *Leucaena* spp. is limited

despite its wide uses. Some information on wood qualities of *L. leucocephala* is available, but little information is available on other species in the genus.

Leucaena. leucocephala is used internationally as a fuelwood. Its calorific value ranges from 4,200 to 4,670 kcal/kg (Brewbaker, 1987a; MacDicken and Brewbaker, 1982), which is comparable to other fast growing, non-resinous hardwoods (Pottinger and Hughes, 1995). The wood features easy splitting and drying, and good burning qualities. Besides direct burning, it makes excellent charcoal with a recovery value between 25 to 30% (Brewbaker, 1987a).

The wood of *L. leucocephala* is strong, heavy, and easy to work with, which makes it suitable for making furniture (Rao, 1984; Van den Beldt and Brewbaker, 1985). But it is of little value for heavy construction because of its low durability and susceptibility to termite attack (Pottinger and Hughes, 1995).

The wood of *L. leucocephala* has excellent pulping qualities, and makes excellent pulping and writing paper (Brewbaker, 1987a). It has higher cellulose and lower lignin contents than other native hardwoods of Taiwan (Tang and Ma, 1982).

Research on mechanical properties, including static bending, compression strength, and toughness indicates that *L. leucocephala* has fair qualities, which would not limit its uses as solid products (Tang, 1981). Van den Beldt and Brewbaker (1985) reported that *L. leucocephala* produced the wood of medium density. Its specific gravity ranges from 0.45 to 0.55 at the age of two years, a value that is fairly comparable to other commonly grown fuelwood species such as *Gliricidia sepium* (MacDicken and Brewbaker, 1982).

Wood qualities of lesser-known Leucaena species

Information on wood properties of lesser-known *Leucaena* species is limited. Nevertheless, some lesser-known species have a long history of wood uses by local farmers, which indicates they may have comparable or higher wood qualities than *L. leucocephala*. In Mexico and Central America, *Leucaena* species are mainly used for fuel and poles, due to their high wood qualities (Hughes, 1998). Among them, *L. collinsii*, *L. lempirana*, *L. magnifica*, *L. salvadorensis,* and *L. trichandra* produce excellent fuelwood. Some species like *L. salvadorensis*, *L. lempirana*, and *L. collinsii* are managed for house construction (Hughes, 1998).

Pottinger et al. (1998) studied the wood properties of 33 accessions of *Leucaena* representing 18 taxa. Wood density and yield were based on samples from three yield trials in Guatamala, the Philippines, and Australia. *Leucaena shannonii, L. collinsii* subsp. *zacapana*, and *L. magnifica* showed the highest wood density across all three sites, while *L. multicapitula* and *L. pulverulenta* had the lowest wood density. *Leucaena leucocephala* had a medium density. Significant intraspecific variation was found in some species, such as *L. esculenta*, *L. pulverulenta*, *L. trichandra*, and *L. trichodes*. Besides wood density, *L. shannonii* and *L. collinsii* subsp. *zacapana* produced the highest wood yield. This study shows a great potential for incorporating lesser-known species into wood production, either by direct uses or by breeding programs.

Characters of Leucaena wood quality

Wood density can imply other wood properties such as compression strength and caloric value (Pottinger et al., 1998; Hughes, 1998). A study on 11 species of *Leucaena*

indicates that caloric values vary little in terms of unit weight of oven-dried wood (Bezkorowajnyj, 1996). Thus wood density can be a main trait to assess overall wood qualities (Hughes, 1998).

Wood qualities of *Leucaena* vary with the age, site conditions, and silvicultural regimes. Spacing and management can influence such factors as the proportion of sapwood to heartwood, stem straightness, the degree of compression, the number and occurrence of knots, and potential end uses of the wood (Van den Beldt and Brewbaker, 1983). Tang and Ma (1982) reported that in *L. leucocephala* population density affects all test properties, including specific gravity (SG), compression strength, and the modulus of elasticity. Their values decreased with the increase of population density from 2,500 to 10,000 stems/ha. The amount of bark increased and the qualities of particleboard decreased with the increase of population density. It was also reported that a close initial spacing helps to produce straight, clear boles (Bhatia and Kapoor, 1985; Rao, 1984). Wood properties, such as density, also vary across tree trunks and between sapwood and heartwood. Thus, a standard assessment procedure is required when comparing different materials (Hughes, 1998).

The relation between wood quality and growth rate is always a central focus to combine high wood qualities with fast growth. The wood qualities of *Leucaena* are not negatively correlated with yield, and there is opportunity to combine high yield with good wood qualities (Pottinger et al., 1998). For example, some accessions of *L. shannonii* show both high wood density and high yield. Stewart et al. (1991) reported that *L. salvadorensis* also produces high wood biomass with high wood density. Hughes (1998)

concluded that it is possible for *Leucaena* species to have both high wood yields and high wood densities.

Why Seedless?

Weediness of Leucaena species and hybrids

The risk of weediness caused by the introduction of *Leucaena* can be of concern. The common genotype of *L. leucocephala* is a wide-spreading and well-known invasive weed and reported as weedy in more than 20 countries. Some interspecific hybrids such as KX3 (*L. leucocephala* x *L. diversifolia*), which is self-fertile and produces abundant seeds, may impose a big environmental risk. Species like *L. diversifolia* also appear to have the invasive traits of *L. leucocephala* and are likely to become weedy (Hughes and Jones, 1998). Most types of *L. leucocephala* and other *Leucaena* spp., however, have not become weedy either domestically or abroad (Brewbaker, personal comm.).

Seedless *Leucaena* hybrids have raised great interest as sterility reduces the risk of weediness (Brewbaker, 1988). This feature is especially useful when *Leucaena* is used for conservation purposes.

History of seedless Leucaena hybrids

First sporadic seedless *Leucaena* hybrid--*L. pulverulenta* x *L. leucocephala* was discovered in the 1940s, and is used as a shade plant in coffee and tea plantations in Indonesia (Lammers, 1940; Brewbaker, 1988). Sporadic seedless hybrids have also been found in other *Leucaena* species. For example, a vigorous and sterile hybrid from the natural cross between *L. esculenta* and *L. leucocephala* was found in Colombia (Hutton

and Eddie, 1982). More seedless hybrids have been obtained from an intense hybridization program conducted by Sorensson (1993), who found that more than 30 out of all 197 interspecific hybrids were sterile or near sterile. Outstanding sterile hybrids such as K1000 (*L. esculenta* x *L. leucocephala*) and the hybrids of *L. pulverulenta* x *L. diversifolia* are currently under evaluation on their growth, wood quality, and adaptation to different environments.

Seedless hybrids are expected to be fast-growing. The seedless hybrid K1000 appeared to outgrow its parents *L. leucocephala* and *L. esculenta* (Sorensson and Brewbaker, 1994; Sorensson, 1995). Fast growth of seedless hybrids can be partially attributed to the concentration of photosynthates on leaf and wood growth rather than on pod and seed development (Brewbaker, personal comm.).

Propagation of seedless hybrids

A reliable propagation system to produce a large amount of plantlets at reasonable cost is needed in order to use seedless hybrids effectively. Seedless hybrid plants can be obtained either from vegetative propagation or from hybrid seeds. A practical method of vegetative propagation of seedless *Leucaena* hybrids has been worked out (Sun, 1996), though the method still needs to be refined, and the cost of production should be further assessed (Sun et al., 1998).

The possibility of producing seeds of seedless hybrids has been explored by several researchers without major breakthrough. Bray and Fullon (1987) conducted a study to produce F1 seeds of *L. pulverulenta* x *L. leucocephala* using self-incompatible diploid species *L. pulverulenta* as seeding parents. However, the results were

disappointing. Only 7 to 29% seeds harvested from *L. pulverulenta* were hybrid. Lower hybrid seed portion might be caused by the breakdown of self-incompatibility due to a pollen mentor effect, which promotes self-incompatible plants to produce remarkable self-pollinated seeds (Sorensson, 1993). Other methods based on self-incompatibility have been proposed by Brewbaker and Sorensson (1990), and Austin et al. (1998), yet the methods have not been extensively studied. Thus, the multiplication of seedless hybrids will more rely on the improvement of the various vegetative propagation methods.

CHAPTER 2. VEGETATIVE PROPAGATION OF *LEUCAENA* HYBRIDS BY CUTTINGS

Introduction

Interspecific hybrids of *Leucaena* have become increasingly significant in *Leucaena* genetic improvement. Outstanding hybrids show high yield of biomass, fast growth, and high resistance to psyllids, apparently due to the combination of characters of parents. One such example is KX2 (*L. leucocephala* x *L. pallida*), which has been intensively evaluated and has displayed great potential for forage in Vietnam and other Asian countries. Another example is K1000 (*L. leucocephala* x *L. esculenta*), which is a seedless triploid, grows fast, and has good wood qualities. The evaluation of various *Leucaena* hybrids is currently underway at the University of Hawaii.

Effective utilization of *Leucaena* hybrids depends on the availability of propagules of the hybrids, which can be obtained from vegetative cuttings, hybrid seed production, or advanced open-pollinated seeds. Several methods have been proposed to produce hybrid seeds (Brewbaker and Sorensson, 1990; Austin et al., 1998). At present, however, no proposed method has effectively produced a large amount of hybrid seeds. Advanced open-pollinated seeds have been produced in fertile KX2, but some researchers still favor F1 plants for fear of losing interspecific heterosis in advanced generations (Speed and Mullen, 2001). Reliable means of vegetative propagation, therefore, are sought for better utilization of potential hybrids and notably of seedless hybrids.

Vegetative propagation is not only needed to multiply potential hybrids, it is also needed for other purposes. Firstly, vegetative propagation can produce clones of self-incompatible species for use as parents for hybrid seed production. Such a procedure has been proposed and

experimented with by several authors (Brennan and Mudge, 1998; Bray and Fullon, 1987). Secondly, uniform clones from single superior self-incompatible trees could be used directly in plantations to reduce the risk of weediness, if they can be cloned economically.

Vegetative propagation has been investigated by many workers in *Leucaena*, especially in *L. leucocephala*. Hu and Liu (1981) succeeded in rooting one-year and three-year old leafy twig cuttings in Taiwan using sand as the medium under a mist system. Bristow (1983) also successfully propagated *L. leucocephala* in Whales by cuttings in chambers covered with polythene and with added bottom heat. In contrast, some researchers reported difficulties in rooting *L. leucocephala* cuttings. Jones et al. (1982) reported that it is difficult to root the cuttings of *L. leucocephala*. Litzow and Shelton (1991) also reported the failure of rooting *L. leucocephala* both in the field and greenhouse with the mist facility, and concluded that *L. leucocephala* is difficult to propagate vegetatively.

Austin (1995) reported on stem cuttings of several *Leucaena* hybrids, including KX2, K1000, and their parents. The overall rooting frequency was low. *Leucaena pallida*, an important parent of KX2, was especially difficult to root. Hybrids KX2, KX3, and K1000 rooted successfully with variable rooting frequencies.

Sun (1996) and Sun et al. (1998) reported large variations in rooting frequencies among hybrids and species, and concluded that rooting frequency of cuttings was correlated with size of leaflets. They also found that cuttings must bear leaves and that bottom-heat and hormone treatments were beneficial for rooting. Dick and Magingo (1998) reported on cutting propagation of *L. leucocephala*. They used single-node cuttings from 1 –year old seedlings, and cuttings with leaves were easier to root than leafless ones. Rooting ability of cuttings also varied among clones and node positions.

Some studies have been devoted to propagate KX2 by cuttings, as it is recognized as an excellent hybrid for forage. Gutteridge et al. (1999) reported a consistent 40 to 65% rooting percentage in KX2 using a technique modified from Sun, who was only able to achieve 10 to 28% of rooting. Le et al. (2001) conducted a series of rooting experiments of KX2 in Vietnam, in which an overall 60% of rooting was achieved. The most suitable cuttings were from 20 to 25-day old stems from coppiced mother plants, and the most suitable rooting compound was 0.75% (w/w) IBA powder.

Rooting systems and plant material used by various researchers are not identical. The results from various sources are, therefore, not fully comparable. Some factors, however, apparently determine the success of rooting. They include 1) leaf presence on the cutting, which is critical for cuttings to root; 2) the age of cuttings--cuttings from young regrown shoots have greater potential to root; 3) rooting compounds, which promote cuttings to root.

Other asexual propagation methods have also been used in *Leucaena*. Bud grafting is routinely used in Indonesia for a seedless hybrid, *L. pulverulenta* x *L. leucocephala*, and the method is described by Brewbaker (1988). Successful grafting is also reported by Zabala (1977), Versace (1982), and Brennan (1992, 1995). Other vegetative propagation methods used in *Leucaena* include the use of large stems (Zabala, 1977; Delton, 1980), foam air-layering (Osman, 1995), and tissue culture (Dhawan and Bhojwani, 1985; Goyal et al., 1985).

The objective of this study was to develop a more reliable cutting propagation method for *Leucaena* hybrids. When cutting propagation methods of *Leucaena* hybrids newly developed by Sun (1996) were first adopted in this research, they seemed

unreliable and did not produce enough propagules for hybrid yield trials. Biological, as well as non-biological factors affecting the rooting ability of *Leucaena* hybrids were explored, and the rooting ability of difficult-to-root hybrids like KX2 was improved.

Methods and materials

General information

All cutting experiments were conducted in the greenhouse at the Waimanalo Research Station. Nine hybrid trees (5 to 16 years old) representing different ploidy levels (2x, 3x, and 4x) and leaflet sizes were selected for the study. The parentages and ploidy levels of the hybrids are listed in Table 2.1. Soft cuttings were taken from young shoots after coppicing. Only soft, green portion of shoots were used for cutting preparation, and highly lignified shoots were discarded. Usually the first one or two nodes of shoots were also discarded. Cuttings in various size and length were pooled and randomly assigned to each replication or treatment. Four (in some cases three) cuttings were potted in two-and-half-inch square pots filled with 1 vermiculite: 1 perlite (v/v) mixture, or 2 perlite: 1 peat moss (v/v) mixture.

Unless specified, all rooting experiments were conducted on shaded (30% shading rate), uncovered 3 x 8 ft benches. The benches were irrigated by a mist system, which turned on for 12 to 14 seconds every 5 minutes in the daytime and 8 seconds every 10 minutes at night. Rooting results were usually inspected after 6 weeks. The number of rooted cuttings, the number of main roots (roots >1cm in length) per cutting, and the length of the longest root were recorded. The first day when the roots penetrated out the bottom hole of the pots was also recorded to indicate the rapidity of root initiation.

Table 2.1. Pedigree and ploidy level of 9 *Leucaena* clonal hybrids used in propagation experiments.

Hybrid	Pedigree	Ploidy level
84-11-1-1	K19 *L. pulverulenta* x K156 *L. diversifolia*	triploid
84-16-4-1	K19 *L. pulverulenta* x K156 *L. diversifolia*	triploid
84-16-4-7	K19 *L. pulverulenta* x K156 *L. diversifolia*	triploid
84-17-4-1	K19 *L. pulverulenta* x K156 *L. diversifolia*	triploid
85-11-1-1	K158 *L. macrophylla*	diploid
85-11-1-2	K158 *L. macrophylla*	diploid
KX3 cl2	*L. leucocephala* x *L. diversifolia*	tetraploid
KX2 cl2	K746 *L. pallida* x K481-W *L. leucocephala*	tetraploid
K1000 cl3	K636 *L. leucocephala* x K 948 *L. esculenta*	triploid

Data from replicated experiments were subjected to analysis of variance (ANOVA) using a spreadsheet developed by Brewbaker (Brewbaker, 2003). All percentage data were transformed by arcsine square root percentage prior to ANOVA.

The mean monthly temperatures of the greenhouse ranged from 22 °C (January) to 27 °C (July). The daylight period ranged from 11 hours (December) to 13 hours (July). Monthly solar radiation (PPFD) ranged from 120 $\mu mol^{-2} s^{-1}$ (December) to 350 $\mu mol^{-2} s^{-1}$ (July). Monthly relative humidity of the greenhouse ranged from 69 to 77%.

Individual experiments

Experiment 1. This experiment was to test the effect of amount of leaf present on the cuttings being rooted. Four levels of leaf presence (0, 1/3, 2/3, and full, ratio of the amount of pinnae left on the cutting to total pinnae, Figure 2.1) were tested in 9 hybrids (Table 2.1) in August 2001. A randomized complete block design with four replications was employed. Each replication had 4 to 8 cuttings. Bi-nodal cuttings were prepared

Figure 2.1. Four levels of leaf presence on bi-nodal K1000 cl1 cuttings. From left to right, cuttings with full, 2/3, 1/3, and 0 leaf presence (ratio of the amount of pinnae left to total pinnae).

from shoots produced 40 days after coppicing. The length of cuttings ranged from 8.5-17.5 cm. The number of cuttings shedding leaves was counted every week after two weeks of cutting insertion.

Experiment 2. In this experiment, the minimum number of pinnae or leaflets of the cutting needed for rooting was investigated in 5 hybrids of K1000 cl3, 84-17-4-1, 84-16-4-7, KX3 cl1, and 85-11-1-1. In August 2001, cuttings were taken from 40-day old shoots after coppicing, and leaves of cuttings were trimmed to 1, 2, 3, 4, and 5 pinnae or 1, 2, 3, and 4 pairs of leaflets before insertion. The experiment was unreplicated with 8 cuttings in each treatment. The numbers of rooted and alive cuttings were inspected after 6 weeks.

Experiment 3. Rooting of one- and two-node cuttings was assessed in 8 hybrids in this experiment in October 2001. A randomized complete block design with four replications was employed. There were eight to twelve cuttings in each replication. Cuttings were from shoots with 40-day regrowth after coppicing, and had entire leaves.

Experiment 4. In this experiment, rooting of cuttings from stock plants coppiced 25, 45, 65, 85, and 105 days before collecting the cuttings was evaluated. Five hybrids, K1000 cl3, KX2 cl2, KX3 cl1, 84-17-4-1, and 84-16-4-1 were tested. A randomized complete block design with three replications was used. There were 8 single-node cuttings in each replication. Usually the main regrown shoots after than 45 days were so lignified that they were not suitable for cuttings, and only side branches were used. The

treatments of 45-day regrowth of KX3 cl1, 25-day regrowth of 84-17-4-1 and 86-16-4-1 were missed because of poor regrowth.

Experiment 5. Rooting of cuttings of the 8 hybrids was conducted in winter (December) of 2001. Bi-nodal cuttings from 40-day regrown shoots were prepared with two levels of leaf presence (1/3 and full) and were replicated four times. There were 8 cuttings in each replication. Data on rooting percentage and the number of main roots per cutting, and the length of the longest root were compared with those from Experiment 1 (summer trial).

Experiment 6. Four rooting systems were tested to determine the most suitable conditions for rooting in winter. The four systems are listed in Table 2.2, and included variants in shade by a covering nylon mesh and variations in surrounding the bench with clear plastic sheets. Bi-cuttings from 40-day regrown shoots of four hybrids were replicated three times with 8 cuttings in each replication. All cuttings were trimmed to half leaves. The four hybrids were K1000 cl3, KX2 cl2, 84-16-4-7, and 84-17-4-1.

Table 2.2. Four rooting systems used for 4 *Leucaena* hybrids in winter.

System	Description
1 (control)	With 30% shading, without plastic covering
2	Without 30% shading, without plastic covering
3	Without 30% shading, with plastic covering
4	30% shading + plastic covering

Experiment 7. In this experiment, etiolation treatments were applied to two hybrids recalcitrant to root, KX2 cl2 and 84-17-4-1. Three consecutive experiments were conducted from March to June of 2003. The stock plants were first coppiced to 60 cm in

height. After new shoots started to sprout, the whole plants were covered with cages that blocked 100% light. The cages were removed after two weeks, and the stock plants were allowed to harden in full sun for 1 week. In the same time, part of etiolated shoots were covered with 6 cm x 1.9 cm Velcro belts below the node during hardening (Figure 2.2). All experiments were replicated three times with 6 to 12 single-node cuttings in each replication. All cuttings were trimmed to half leaves.

Figure 2.2. Application of Velcro belts in KX cl2 during hardening after etiolation. Velcro belts were applied below the nodes.

Results

Leaf presence on the cuttings (Experiment 1)

Effect of genotype

Rooting ability of *Leucaena* hybrids was basically affected by the genotype of hybrids (Table 2.3). Most hybrids rooted except hybrid 84-11-1-1, which had not produced any roots by 6 weeks. Rooting frequencies of the other 8 hybrids ranged from 19.8 to 88.5 %. The mean numbers of main roots per cutting ranged from 2.7 to 12.2. The mean length of the longest root ranged from 3.6 to 12.5 cm. The 9 hybrids could be roughly grouped into 4 classes based on rooting frequency and rooting qualities. Group 1 included, 85-11-1-1 84-16-4-7, and 85-11-1-2, which had the highest rooting frequencies (80.2, 88.5 and 88.4%, respectively), largest number of main roots per cutting, and longest length of the longest root. Group 2 included 84-16-4-1, KX3 cl1, and K1000 cl3, which had medium rooting frequencies (59.4, 64.6, and 77.1%, respectively) and numbers of main roots per cutting. Group 3 included KX2 cl2 and 84-17-4-1 and had only low rooting frequencies (19.8 and 25.0%). Group 4, which included 84-11-1-1, did not produce any roots under existing circumstances. ANOVA revealed that there were significant differences of rooting frequency, the number of main roots per cutting, and the length of the longest root among 8 hybrids that produced roots.

Table 2.3. Effects of the amount of leaf present on the cutting on rooting frequency, number of main roots per cutting, and length of the longest root of 8 hybrids.

	Rooting frequency (%)	Number of main roots per cutting	Length of the longest root (cm)
Hybrid (H)			
84-11-1-1	0.0 †	0.0 †	0.0 †
KX2 cl2	19.8 a‡	2.7 a	3.6 a
84-17-4-1	25.0 a	4.6 a	6.0 b
84-16-4-1	59.4 b	7.9 b	7.9 bc
KX3 cl1	64.6 be	9.5 b	9.0 cd
K1000 cl3	77.1 bef	7.7 b	--
85-11-1-1	80.2 cef	8.2 b	10.6 de
84-16-4-7	88.4 cf	12.2 c	11.6 e
85-11-1-2	88.5 c	11.4 c	12.5 e
Leaf (L)			
0 leaf	0.0 †	0.0 †	0.0 †
1/3 leaf	56.4 a	5.1 a	7.1 a
2/3 leaf	66.0 b	9.1 b	9.5 b
full leaf	66.2 b	9.9 b	9.6 b
Significance			
Hybrid	**	**	**
Leaf	*	**	**
H x L	NS	NS	NS

NS, *, and **: Nonsignificant or significant at $p<0.05$ and 0.01, respectively.
†: Indicating that the data were not included in ANOVA because no cuttings rooted in those treatments.
‡: Means with the same letter are not significantly different.

Amount of leaf present on the cutting

Leaf presence on the cutting was essential for rooting of soft cuttings. All cuttings without leaves failed to root (Table 2.3). The amount of leaf presence affected rooting frequency, the number of main roots per cutting, the length of the longest root, and days for root initiation. Cuttings with 2/3 or entire leaf rooted better than those with only 1/3 leaf (66% and 66.2% vs. 56.4%), had better root qualities (Table 2.3, Figure 2.3), and needed fewer days for root initiation (25 and 24 days vs. 28days). There was no interaction between hybrid and leaf presence in terms of rooting frequency, the number of main roots per cutting, and the length of the longest root, suggesting that different hybrids had the same rooting response to leaf presence on cuttings (Table 2.3).

Figure 2.3. Rooted cuttings of 2 *Leucaena* hybrids with four levels of leaf presence on the cutting. Cuttings left with larger portion of leaf had better root qualities. No cuttings without leaves rooted. A: 85-11-1-1. B: 84-16-4-1.

Correlation between leaf shedding and cutting death

The leaflets of cuttings started to shed around 2 weeks after insertion. Leaf shedding of most hybrids increased after 4 weeks and reached a climax in 5 weeks. The number of cuttings shedding leaves after 4 weeks was highly correlated with the number of dead cuttings ($r=0.82$, $p<0.05$). Such a correlation can be explained by the fact that roots usually initiated around this time, and those cuttings shedding leaves would die. It seems that leaf retention for at least 4 weeks was critical for rooting of cuttings.

Leaf retention was important not only for root initiation, but also for root development. Cuttings without leaf shedding developed fibrous roots, but those shedding leaves failed to develop fibrous roots (Figure 2.4 A). Cuttings shedding leaves often died even if they had initiated roots (Figure 2.4 B). Cuttings that lost original leaves and had new leaves usually failed to root. It was evident that the original leaves of cuttings played a critical role in root initiation and root development. Despite the importance of leaf retention to cuttings, it is still unclear if the leaf shedding was the cause or consequence of rooting failure. Leaf shedding usually followed the swelling of lenticel cells and might be signaled by physiological state of the stem, which was determined by genetics as well as by environmental factors such as watering time and light intensity. Leaf shedding thus might be the consequence, not the cause of rooting failure.

Figure 2.4. A: Fibrous roots were produced on cuttings that retained the leaves, and no fibrous roots were produced on cuttings dropping the leaves during rooting. B: Rooted cuttings died after leaf shedding.

Minimum leaflets needed for rooting of cuttings (Experiment 2)

Table 2.4 indicates that for most hybrids the minimum pinnae or leaflets needed for cuttings to root was 2 or 3 pinnae, or two pairs of leaflets (for hybrid 85-11-1-1 with large leaflets). Cuttings with few leaflets did not root even if they were still alive. Cuttings with only minimum numbers of leaflets often tended to be less successful in rooting, since any loss of leaflets led to rooting failure. As suggested by Experiment 1 and 2, as many leaflets as possible should be left on cuttings to achieve rapid and stable rooting, as well as better root qualities.

Table 2.4. Minimum leaflets needed for rooting of cuttings of 5 *Leucaena* hybrids†

		Cuttings with				
Hybrid		1pinna	2 pinnae	3 pinnae	4 pinnae	5 pinnae
K1000 cl3	# of rooted	1	5	5	4	3
	# of alive	4	2	2	3	0
84-17-4-1	# of rooted	0	0	2	4	1
	# of alive	1	1	0	0	0
84-16-4-7	# of rooted	1	2	7	2	8
	# of alive	0	1	0	1	0
KX3 cl1	# of rooted	0	4	5	6	8
	# of alive	6	1	2	0	0
85-11-1-1	# of rooted	1	6	8	4	--
	# of alive	6	1	0	3	--

†: All treatments had eight cuttings. For hybrid 85-11-1-1, different pairs of leaflets instead of pinnae were tested due to its large leaflets.

One- and two-node cuttings (Experiment 3)

Experiment 3 in Oct. 2001 involved 1- and 2- nodal cuttings of 8 hybrids. The results are presented in Table 2.5. Overall rooting frequency and root qualities were inferior to those of Experiment 1 (Table 2.3) perhaps due to cold weather of October and November that affected the rooting of cuttings. The overall rooting frequency of 1-node and 2-node cuttings was 45.8 and 50.1% respectively. The overall number of main roots

per cutting of 1-node and 2-node cuttings was 7.3 and 7.7 respectively. The overall length of the longest root of 1-node and 2-node cuttings was 9.0 and 9.1 cm respectively. No significant differences were found in rooting frequency, number of main roots per cutting, and length of the longest root between one and two-node cuttings. Cuttings with one or two nodes clearly had similar rooting ability.

Table 2.5. Effects of node number of cuttings on rooting frequency, number of main roots per cutting, and length of the longest root of 8 hybrids.

	Rooting frequency (%)	Number of main roots per cutting	Length of the longest root (cm)
Hybrid (H)			
KX2 cl2	19.0	2.9	2.9
84-17-4-1	32.1	5.2	6.6
KX3 cl1	49.9	11.4	7.4
85-11-1-1	50.6	6.7	13.2
84-16-4-7	52.4	8.0	10.0
K1000 cl3	52.6	8.0	9.7
84-16-4-1	60.8	7.2	7.6
85-11-1-2	66.3	10.6	14.8
No. of node (N)			
1 node	45.8	7.3	9.0
2 nodes	50.1	7.7	9.1
Significance			
Hybrid	**	**	**
No. of nodes	NS	NS	NS
H x N	NS	NS	NS

NS, *, and **: Non-significant or significant at $p<0.05$ and 0.01, respectively.

Regrowth days of shoots (Experiment 4)

Five hybrids were cloned at various stages of regrowth after coppicing in Experiment 4. Regrown shoots older than 45 days were usually highly lignified with reddish color, whereas 25-day old shoots were soft and green. Results of the experiment are presented in Table 2.6. Regrowth days greatly influenced the rooting ability of most hybrids. Cuttings of younger shoots rooted better. Cuttings from 25-day regrown shoots rooted best among all cuttings in the hybrids of K1000 cl3 and KX2 cl2. It seemed that

45 to 65 days of regrowth was a critical regrowth period for most hybrids, beyond which cuttings had very low rooting frequencies and poor root qualities. Only cuttings of hybrid KX3 cl1 rooted equally well at all regrowth ages. Cuttings of hybrids 84-17-4-1 rooted poorly at all 4 regrowth periods. It seemed unlikely to improve the rooting ability of those difficult-to-root hybrids such as KX2 and 84-17-4-1 significantly in terms of days of regrowth.

ANOVA revealed that there were significant differences ($p<0.0001$) in rooting frequency, number of main roots per cutting, and length of the longest root among five hybrids and five periods of regrowth. No significant interaction of hybrid and regrowth period was found (Table 2.6).

Table 2.6. Effects of regrowth days on the rooting of cuttings of 5 *Leucaena* hybrids.

Hybrid	Days of regrowth	Rooting Frequency (%)	Number of main roots per cutting	Length of the longest root (cm)
K1000 cl3	25	95.8	15.0	10.8
	45	87.5	13.3	10.1
	65	25.0	3.3	3.5
	85	37.5	3.6	4.0
	105	8.3	0.8	1.5
KX2 cl2	25	33.3	5.4	4.5
	45	20.8	1.8	1.9
	65	0.0	0.0	0.0
	85	0.0	0.0	0.0
	105	0.0	0.0	0.0
KX3 cl1	25	95.8	19.7	12.2
	45	100.0	19.2	11.7
	65	58.3	13.5	11.5
	85	-	-	-
	105	91.7	17.1	10.3
84-17-4-1	25	-	-	-
	45	14.3	1.7	1.6
	65	0.0	0.0	0.0
	85	12.5	2.2	2.2
	105	0.0	0.0	0.0
84-16-4-7	25	-	-	-
	45	41.3	11.8	4.4
	65	49.4	9.2	5.7
	85	29.2	7.6	2.9
	105	8.3	2.8	1.7
Significance				
Hybrid (H)		$p<0.0001$	$p<0.0001$	$p<0.0001$
Days of regrowth (D)		$p<0.0001$	$p<0.0003$	$p<0.0001$
H x D		NS	NS	NS

Rooting in winter and summer (Experiment 5)

Experiment 5 examined rooting ability of 8 hybrids in December, under much cooler temperatures (Table 2.7). The mean temperature of December and August in Waimanalo greenhouse was 22.8 and 26.8 ^{o} C respectively. The light intensity of December and August in the greenhouse was 120 and 313 $\mu mol^{-2} s^{-1}$ respectively. All hybrids had lower rooting frequencies and poorer root qualities in winter than in summer. Some hybrids such as K1000 cl3 and KX2 cl2 did not root in winter. The reduction of the overall average of rooting frequency was 42.8% (18.5% in winter vs. 61.3% in summer). The overall number of main roots per cutting decreased by 4.0 (2.5 roots in winter vs. 7.5 roots in summer), and the overall length of the longest root decreased by 3.6 cm (3.7 cm in winter vs. 7.3 cm in summer). The results were similar to Sun's work (Sun, 1996), where all three of his rooting experiments on K1000 and K1001 in winter failed, whereas they rooted well in summer's experiments.

Different hybrids responded to seasons differently. K1000 cl3 rooted in summer very well with the highest rooting frequency of 90%, but did not root in winter. On the other hand, some hybrids such as 85-11-1-1 were less sensitive to seasonal change. Such differences of rooting response to season among hybrids appeared to be genetic.

ANOVA revealed that there were significant differences ($p<0.0001$) in rooting frequency, number of main roots per cutting, and length of the longest root among 8 hybrids, two levels of leaf presence, and two seasons (Table 2.7). Significant interaction of hybrid and season was found (Table 2.7) with evidence that different hybrids responded to the seasons differently.

Table 2.7. Comparison of rooting of cuttings of 8 hybrids with 2 levels of leaf presence in summer and winter.

Hybrid	Leaf presence	Rooting frequency (%)		Number of main roots per cutting		Length of the longest root (cm)	
		Summer	Winter	Summer	Summer	Summer	Winter
K1000 cl3	1/3 leaf	68.8	0.0	4.4	0.0	-	0.0
	Full leaf	84.4	4.2	9.2	0.4	-	4.2
KX2 cl2	1/3 leaf	9.4	0.0	1.3	0.0	3.3	0.0
	Full leaf	25.0	0.0	4.0	0.0	4.8	0.0
KX3 cl1	1/3 leaf	62.5	0.0	8.2	0.0	8.4	0.0
	Full leaf	56.3	9.4	9.7	3.2	9.5	2.1
84-17-4-1	1/3 leaf	12.5	0.0	1.3	0.0	1.8	0.0
	Full leaf	34.4	0.0	7.2	3.0	9.0	1.3
84-16-4-1	1/3 leaf	56.3	5.2	5.5	1.0	6.9	1.0
	Full leaf	71.9	7.3	9.2	3.8	8.2	2.3
84-16-4-7	1/3 leaf	81.3	15.6	9.0	1.5	8.5	2.2
	Full leaf	93.3	37.5	15.4	3.8	12.2	5.8
85-11-1-1	1/3 leaf	68.8	62.5	5.5	4.8	9.2	10.1
	Full leaf	78.1	54.2	9.9	5.8	11.2	9.4
85-11-1-2	1/3 leaf	91.7	37.5	6.0	6.6	11.6	11.6
	Full leaf	86.5	62.5	14.1	6.3	12.7	8.5
Overall		61.3	18.5	7.5	2.5	7.3	3.7
Significance							
Hybrid (H)		p<0.0001		p<0.0001		p<0.0001	
Leaf (L)		p<0.0001		p<0.0008		p<0.0008	
Season (S)		p<0.0001		p<0.0001		p<0.0001	
H x S		p<0.0001		p<0.0005		p<0.0038	
L x S		NS		p<0.0003		NS	
H x T		NS		NS		NS	

Different rooting systems in winter (Experiment 6)

Experiment 6 tested the effects of three modified rooting systems on rooting of 4 *Leucaena* hybrids in cold winter. The results were given in Table 2.8. No cuttings rooted in system 4 (with 30% shading and plastic covering). The other three systems gave the similar rooting frequencies, but different root qualities (Table 2.8). There were no statistically significant differences among the three systems with rooted cuttings. The rooting frequencies and root qualities were comparable to those of Experiment 5 (rooting in winter). The system 2 (without 30% shading and without plastic covering) and 3

(without 30% shading, with plastic covering) were expected to increase light intensities and temperatures in winter. However, rooting in these two systems was not improved as expected. The results indicated that such increases in temperatures and light in modified benches were still not enough for rooting of cuttings since the temperatures and light intensities were so low in the greenhouse in winter. It was also possible that there were other factors such as physiological (weak regrown shoots) and environmental factors (strong wind) limiting rooting in winter.

Table 2.8. Rooting of cuttings of 4 *Leucaena* hybrids in 4 rooting systems in winter.

	Rooting frequency (%)	Number of main roots per cutting	Length of the longest root (cm)
Rooting System‡			
System 1	21.2	6.2	6.3
System 2	17.7	4.2	5.6
System 3	17.0	3.3	4.1
System 4	0.0‡	0.0‡	0.0‡
Hybrid			
K1000 cl3	27.8	10.0	9.7
KX2 cl2	6.9	1.0	3.2
84-16-4-7	33.3	5.5	4.4
84-17-4-1	6.5	1.8	4.2
Significance			
System (S)	NS	NS	NS
Hybrid (H)	**	**	**
S x H	NS	NS	NS

‡: System 1: with 30% shading, without plastic covering, control. System 2: without 30% shading and without plastic covering. System 3: without 30% shading, with plastic covering. System 4: with 30% shading and plastic covering.
‡: Indicating that the data were not included in ANOVA because no cuttings rooted in those treatments.
NS and **: Non-significant difference and significant at $p<0.01$.

Etiolation treatments (Experiment 7)

Etiolation treatments

Two etiolation treatments, etiolation and etiolation plus Velcro belts, were applied to two difficult-to-root hybrids, KX2 cl2 and 84-17-4-1 from March to July of 2003. New shoots produced in dark had normal sizes of diameter and lengths of internode, but had a pale-yellow color. Etiolated shoots became green quickly after exposure to sun. The rooting results of KX2 cl2 are summarized in Table 2.9. Cuttings of KX2 cl2 from etiolation treatments had significantly higher rooting frequencies than those from the controls in all three consecutive experiments. Etiolated cuttings also had better root qualities than the controls. Duncan multiple range tests revealed there were no significant differences in rooting frequency and root qualities between the two etiolation treatments.

Rooting results of hybrid 84-17-4-1 are summarized in Table 2.10. Cuttings from etiolation treatments rooted significantly better than those from the controls in all three consecutive experiments, evidenced by higher rooting frequencies and better root qualities. Duncan multiple range tests revealed that cuttings treated with etiolation + Velcro belts had significantly higher rooting frequencies and numbers of main roots per cutting than those treated with etiolation alone, but there were no differences in length of the longest root between the two etiolation treatments.

Table 2.9. Effects of etiolation on rooting frequency, number of main roots per cutting, and length of the longest root of hybrid KX2 cl2 cuttings.

	Rooting frequency (%)	Number of main roots per cutting	Length of the longest root (cm)
Experiment (Exp)			
1	47.5 a†	5.4 a	5.5 a
2	74.7 b	6.2 a	5.4 a
3	69.4 b	6.2 a	5.2 a
Treatment (T) ‡			
Etiolation	76.7 a	7.3 a	6.4 a
Etiolation+Velcro	80.7 a	6.6 a	5.6 a
Control	34.4 b	4.1 b	4.1 b
Significance			
Experiment	NS	NS	NS
Treatment	**	**	*
Exp x T	NS	**	**

NS, *, and **: Non-significant or significant at $p<0.05$ and 0.01, respectively.
‡: Etiolation: Cuttings developed under 2 weeks of etiolation and 1 week of greening period. Etiolation + Velcro: Cuttings developed under 2 weeks of etiolation and 1 week of Velcro belts under sun.
†: Means with the same letter are not significantly different.

Table 2.10. Effects of etiolation on rooting frequency, number of main roots per cutting, and length of the longest root of hybrid 84-17-4-1 cuttings.

	Rooting frequency (%)	Number of main roots per cutting	Length of the longest root (cm)
Experiment (Exp)			
1	10.2 a†	2.0 a	2.9 a
2	36.9 b	5.7 b	4.1 b
3	51.1 c	7.2 c	11.5 c
Treatment (T)‡			
Etiolation	36.4 a	5.9 a	7.7 a
Etiolation+Velcro	56.2 b	7.4 b	7.6 a
Control	5.6 c	1.7 c	3.2 b
Significance			
Experiment	**	**	**
Treatment	**	**	**
Exp x T	NS	NS	NS

NS,*, and **: Non-significant or significant at $p< 0.05$ and 0.01, respectively.
‡: Etiolation: Cuttings developed under 2 weeks of etiolation and 1 week of greening period. Etiolation + Velcro: Cuttings developed under 2 weeks of etiolation and 1 week of Velcro belts under sun.
†: Means with the same letter are not significantly different.

Discussion

Rooting ability of *Leucaena* hybrids basically depended on genotypes. Different hybrids consistently showed dramatic differences in rooting percentage and root qualities. The results were in accordance with those of Austin (1995) and Sun (1996), who concluded that K1000 is a hybrid that is easy to root, whereas hybrids like KX2 and species like *L. pallida* are difficult to root. The variability of adventitious rooting has been reported in many forest species such as *Pinus taeda* L. (Foster, 1990), *Eucalyptus globulus* Labill. (Borralho and Wilson, 1994), and *Populus balsamifera* (Farmer et al., 1987). Both additive and non-additive effects reportedly control the inheritance of rooting ability. Variation in rooting ability not only existed among hybrids from different crosses, it occurred among hybrids from the same crosses. For example, four hybrids from the cross of *L. diversifolia* x *L. pulverulenta* displayed great variability in rooting ability.

Leaf presence on cuttings was essential for root initiation of cuttings of *Leucaena* hybrids in the mist system. The importance of the presence a large portion of leaf on the cutting has not been explicitly stressed by other researchers. Sun (1996) suggested keeping one pair of pinnae for K1000 cuttings. However, in our experience, cuttings with such small portion of leaf usually did not root well. This might explain the failure of rooting at the beginning of this study, although the exact rooting procedure of Sun was adopted. Some authors like Dick and Magingo (1998) reported that leafless cuttings of *L. leucocephala* could produce roots in a non-mist system, but the rooting percentage was very low. In a mist system leafless cuttings will never produce any roots as showed in our study because of the rotting of cuttings. The benefit of leaving a large portion of leaf

on the cutting was manifest in our study. More leaf left on cuttings led to higher rooting percentages, better root qualities, quicker root initiation, and more reliable outcomes.

Leaf shedding during rooting was observed frequently. Cuttings of difficult-to-root hybrids shed leaves earlier than those of easy-to-root ones, a phenomenon also found in avocado, where the cuttings of some difficult-to-root varieties shed leaf earlier than those of easy-to-root varieties (Reuveni and Raiviv, 1980). It is unclear if leaf shedding was the cause or the consequence of rooting failure. It seems that it was the consequence of rooting failure. Some difficult-to-root hybrids such as KX2 cl2 could sustain leaf retention for a long time (30 days), a period seemingly long enough for root initiation. However, its cuttings rooted poorly in this study.

Node numbers of cuttings did not affect the rooting of cuttings of *Leucaena* hybrids. No authors have explicitly stated the suitable node number for *Leucaena* cuttings based on experiments. Sun (1996) suggested using bi-nodal cuttings in K1000. Our results in Experiment 3 showed that single-node cuttings rooted as well as bi-nodal cuttings in all hybrids studied. In the future, using single-node cuttings can double the number of cuttings from the same amount of stock plants.

Some hybrids like K1000 still remained problematic in rooting in winters. There are several probable causes of the problem. Firstly, low temperatures and low light intensities in Hawaii's winters might be the main causes. At the Waimanalo greenhouse, the average temperature in winter is about 5 $^{\circ}$C lower than that in summer (22 $^{\circ}$C vs. 27 $^{\circ}$C), and the light intensity in winter is only one-third of that in summer (120 $\mu mol^{-2} s^{-1}$ vs. 350 $\mu mol^{-2} s^{-1}$). Secondly, the physiological state of cuttings in winter was usually not as good as that in summer. Plant regrowth after coppicing in wintertime was slower

and less vigorous than in summertime, and psyllid infestation occurred constantly in winter. Psyllid infestation on young shoots and leaves of all the hybrids caused leaf damage after young leaves unfolded. It was observed that cuttings tended to shed leaves earlier after they were infested by psyllids, evidently contributing to lower rooting frequencies. The possible remedies of poor rooting in wintertime include light supplement and bottom-heat to cuttings. The benefit of bottom-heat for rooting of *Leucaena* cuttings has been proved in Sun's work (Sun, 1996). Improved rooting in winter should also include the control of psyllid infestation.

The age of coppiced shoots affected the rooting of cuttings. For most hybrids, cuttings from younger shoots rooted better than those from older ones. Le et al. (2001) suggested using 20 to 25-day old shoots in propagation of KX2. Our results were identical to their conclusion. Younger cuttings would be less lignified and lower in phenolics, facilitating root initiation.

Rooting ability of cuttings was significantly increased by etiolation treatments in two difficult-to-root hybrids, KX2 cl2 and 84-17-4-1. Etiolation is often used to improve rooting ability of cuttings of temperate species (Hartmann and Kester, 2002). The method has been successfully used in temperate species such as *Carpinus betulus* (Maynard and Bassuk, 1992), *Syringa vulgaris* (Howard and Harrison-Murray, 1995), and other woody species (Maynard and Bassuk, 1987). It has not been used frequently in tropical species. The easy and rapid regrowth of *Leucaena* makes it suitable for the etiolation treatments. Although we tested the etiolation treatments only in two *Leucaena* hybrids, remarkable improvement in rooting ability of cuttings leads us to believe that the

treatments might be also effective in other difficult-to-root hybrids and species, like *L. pallida*, *L. esculenta*, and *Acacia koa*.

Conclusions

Rooting ability of *Leucaena* hybrids basically depended on genotype. Cuttings required leaf presence for reliable rooting. Large leaf segment led to higher rooting percentages and better rooting quality. One-node cuttings had the same rooting ability as bi-nodal cuttings. Cuttings from earlier regrowth following coppicing rooted better than older ones. Rooting ability of the hybrids decreased dramatically in cold, low-light seasons. Etiolation treatments remarkably increased the rooting ability of two difficult-to-root hybrids in summer. Future work should be focused on improving rooting ability of the hybrids in cold seasons by using supplemental light and bottom heat.

CHAPTER 3. YIELD TRIALS OF *LEUCAENA* HYBRIDS

Introduction

The hybridizations among *Leucaena* species have produced a large number of hybrids with broadened adaptability and potential uses. Hybrids between *L. leucocephala* and other lesser-known species are especially of interest to researchers, since those hybrids retain more or less good characters of *L. leucocephala* while some unique characters such as resistance and tolerance to environmental stresses can be added. The examples of such hybrids are KX3 (*L. leucocephala* x *L. diversifolia*), which thrives at high elevations (850m) where *L. leucocephala* does not grow well (Brewbaker and Sorensson, 1990) and KX2, which has high resistance to psyllids, high forage yield, and high fodder quality, as proved in extensive trials.

Other interesting interspecific hybrids are triploid hybrids. A well-known one is K1000 (*L. leucocephala* x *L. esculenta*). It is a triploid hybrid, which grows rather fast and is highly resistant to psyllids. It is seedless due to the abnormality of chromosome pairing (Hutton and Eddie, 1982). A reason for its fast growth might be due to its seedlessness, given more energy of plants can be diverted to the growth of stem instead of fruits (Brewbaker, personal comm.). The seedlessness itself is an attractive merit, since the risk of weediness posed by some varieties of *L. leucocephala* and tetraploid hybrids like KX3 is avoided. The hybrid K1000 also seems to have good wood qualities. Its wood is heavy and dark colored (personal observations). There is major potential to grow this hybrid for high-value hardwood. A fast-growing, environment-benign, and

widely adaptive hybrid like K1000 may also provide a good opportunity for soil bioremediations and environment conservations.

The objective of this study was to investigate the growth rates and growth characters of K1000 hybrids, compared to other *Leucaena* hybrids at three locations.

Materials and methods

Entries

Clones of 26 hybrids and seedlings of two controls, K636 and KX2 (seeds) were included in the study. The code, pedigree, and ploidy level of the entries are listed in Table 3.1.

Waimanalo trial (SET 01-4)

Twelve of listed hybrids plus two controls were included in the trial at Waimanalo, Oahu. Among the 12 hybrids, 6 were triploid, 3 tetraploid, and 3 diploid. K636 and KX2 (seeds), both widely used in plantations, were used as controls. The trial was planted in June 25, 2001. All trees were propagated at the same time, and uniform plants were selected for planting.

The trial was replicated three times with 5 trees in each plot. The trees were planted at 2 x 1.5 m spacing (2 m within plots and 1.5 m between plots). Border trees were planted around three sides of the field. Drip irrigation was applied in first 12 months. No fertilizer was applied after planting. Early weed control was done under standard management. DBH, height, and the number of main stems were measured every 6 months. Psyllid damage was also observed.

Table 3.1. Pedigree and ploidy level of 28 entries involved in three yield trials at Kona (SET 01-2), Hamakua (SET 01-3), and Waimanalo (SET 01-4)

Entries	Pedigree	Ploidy level	Trial
85-7-1-1	K75-1*L. pulverulenta* x K450 *L. collinsii*	2N	SET 01-3
85-7-1-8	" " " " "		SET 01-3
85-11-1-1	K158 *L. macrophylla* sib	"	SET 01-3, 01-4
85-11-1-2	" " "	"	SET 01-3, 01-4
85-15-1-11	K409 *L. trichandra* x K156 *L. diversifolia*	3N	SET 01-3, 01-4
84-16-4-1	K19 *L. pulverulenta* x K156 *L. diversifolia*	"	SET 01-3, 01-4
84-16-4-7	" " " " "	"	SET 01-3, 01-4
84-17-4-1	" " " " "	"	SET 01-3, 01-4
85-9-3-8	*L. diversifolia* x *L. pulverulenta*	"	SET 01-3
85-9-3-9	" " "	"	SET 01-3
K1000 cl2	K636 *L. leucocephala* x K 838 *L. esculenta*	"	SET 01-3, 01-4
K1000 cl3	K636 *L. leucocephala* x K 948 *L. esculenta*	"	SET 01-2, 01-3, 01-4
K1000 wcl1	K636 *L. leucocephala* x K 838 *L. esculenta*	"	SET 01-3
K1000 wcl2	" " " " "	"	SET 01-3
K1000 wcl3	" " " " "	"	SET 01-3
K1000 wcl4	" " " " "	"	SET 01-3
K1000 wcl5	" " " " "	"	SET 01-3
K1000 wcl6	" " " " "	"	SET 01-3
K1000 wcl7	" " " " "	"	SET 01-3
K1000 wcl8	" " " " "	"	SET 01-3
K1000 wcl9	" " " " "	"	SET 01-3
K156 x K376	*L. diversifolia* x *L. pallida*	4N	SET 01-3, 01-4
KX3 cl1	K636 *L. leucocephala* x K156 *L. diversifolia*	"	SET 01-3, 01-4
KX3 cl2	" " " " "	"	SET 01-3, 01-4
KX2 cl1	K806 *L. pallida* x K636 *L. leucocephala*	"	SET 01-3
KX2 cl2	K746 *L. pallida* x K481-W *L. leucocephala*	"	SET 01-3, 01-4
K636	*L. leucocephala*	"	SET 01-2, 01-3, 01-4
KX2 (seeds)	*L. pallida* x *L. leucocephala*	"	SET 01-2, 01-3, 01-4

The Waimanalo Research Station is located at 21° 20' N, 158° 20' W with a mean elevation of 20 m above sea level. Its annual precipitation averages 1380 mm, and the mean annual temperature is 24.6 °C. Total precipitation measured during the experimental period (June 2001 to December 2002) was 1020 mm, about half of normal.

Hamakua trial (SET 01-3)

Twenty-six hybrids and the two controls, K636 and KX2 (seeds), as listed in Table 3.1, were used at Hamakua, Hawaii Island. Young trees were propagated in June

of 2001 at Waimanalo at the same time, and uniform plants were selected for planting. They were transplanted in 2''x 2'' square pots or in 1.5'' dibble tubes from rooting medium one and half months before planting. The trial was planted on October 22, 2001. It was replicated 3 times with 5 trees in each replication. The spacing of trees was 1.5 x 2 m (1.5 m within plots and 2 m between plots). No border trees were planted. Due to the lack of adequate trees, some entries had only one or two replications. Drip irrigation was applied in the first 6 months to aid early establishment. After 6 months of establishment, dead trees were recoded and replaced. Since the initial growth at Hamakua was slow, the first measurement of height and DBH was taken at the age of 15 months, and the second one at the age of 21 months.

The Hamakua Research Station is located at 19° 58' N and 159° 23' W with a mean elevation of 650 m above sea level. Its precipitation ranges widely from 1500 to 2300 mm per year, and mean annual temperature is 16° C. The rainfalls of 2001 and 2002 were 2075 and 2575 mm, respectively.

Kona trial (SET 01-2)

This is a small demonstration trial that only included K1000 cl3 and the two controls, K636 and KX2 (seeds). The trial was located at Kona Research Station, Hawaii Island and planted in March 2001. It was replicated twice with 5 trees in each replication. The spacing of trees was 2 x 2 m. No drip irrigation was applied. DBH and height were measured in December 2002.

The Kona Research Station is located at 19° 32' N and 156° 56' W with a mean elevation of 420 m above sea level. Annual precipitation is 1500 mm, and mean annual

temperature is 21.7 ° C. The rainfalls of 2001 and 2002 were 1261 and 1036 mm respectively.

Statistical methods

Mean DBHs of trees with multiple-stems were calculated by:

$DBH=\sqrt{(DBH_1^2+DBH_2^2+\ldots+DBH_n^2)}$

where DBH_1, DBH_2, ... ,DBH_n were the DBHs of stems in each tree.

Wood volume (V) was calculated by:

$V=0.5 \times D^2 \times H$, where D is the DBH of the tree, and H is the height of the tree (Van den Beldt, 1983).

The analysis of variance (ANOVA) based on single-tree data was conducted on the SAS® system by "PROC GLM". Because of missing trees, only type III sum squares were interpreted. Correlations were calculated by SAS "PROC CORR". Mean separations were done by using the least significant difference (LSD, p=0.05).

Results

SET 01-4, Waimanalo, Oahu

Overall survival rates of the trial at the ages of 6, 12, and 18 months were 99.1, 99.1, and 96.2% respectively. Generally, all the entries survived very well with the help of drip irrigation. However, almost all trees suffered from wild pig damage on low bark around 6 months after planting. Most of injury was not fatal, and most trees recovered quickly from the injury. Only a few trees were seriously injured and failed to recover.

No subsequent damage was found. No major psyllid damage was observed in the Waimanalo trial due to a small psyllid population there.

Most hybrid trees were blown over by storms during the winter of first year (2001), whereas no trees of the two controls, which were raised from seeds, were blown over (Figure 3.1). Those blown-over trees did not further lean down in the second winter, partially because the irrigation was cut, which might have induced deep growth of root systems.

Average height, DBH, wood volume, and number of main stems of 14 entries at different ages are summarized with LSD at p=0.05 level in Table 3.2. Significant differences in growth among the entries were found. The overall mean of the height of the entries was 3.2, 5.9, and 6.2 m at the age of 6, 12, and 18 months respectively. Trees grew extremely fast in the first 12 months with the help of drip irrigation, and the increment of height between the age of 12 and 18 months dramatically decreased, partly due to the absence of irrigation.

Trees gained an overall mean of 50.5 and 58.1 mm of DBH at the ages of 12 and 18 months respectively. The overall mean of the number of main stems was 2.8 and 1.8 at the ages of 12 and 18 months respectively, and the overall mean of wood volume was 9.1 and 12.5 dm^3 at the ages of 12 and 18 months respectively.

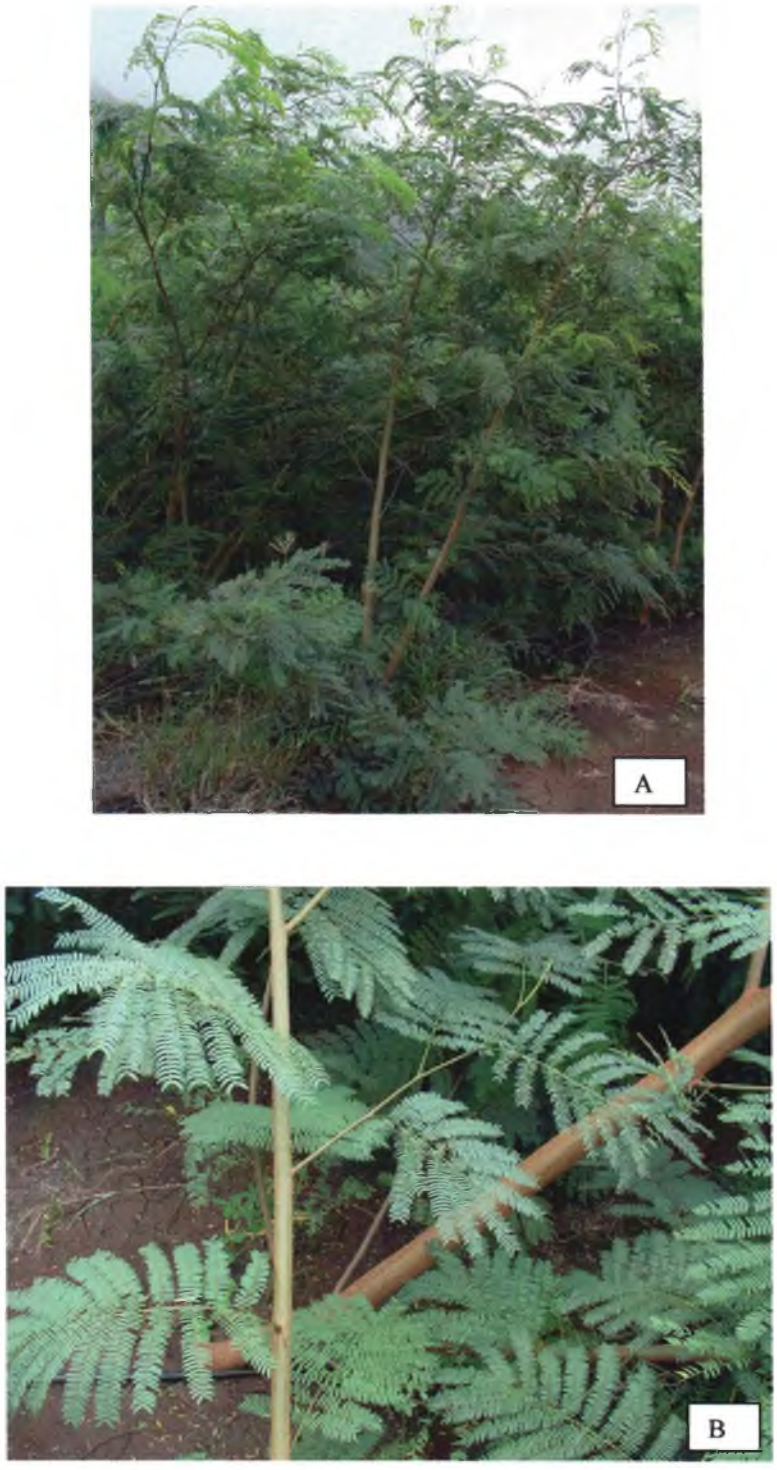

Figure 3.1. Trees raised from seeds remained erect (A) while most trees from cuttings were blown over in stormy winter (B) at Waimanalo, Oahu.

Table 3.2. Average tree height, diameter at breast height (DBH), number of main stems, and wood volume after 18 months growth of *Leucaena* hybrids at Waimanalo, Oahu (SET 01-4).

	Height (m) at the age (months)of			DBH (mm) at the age (months) of		# of branches at the age (months) of		Wood volume (dm^3) at the age (months) of	
Entries	6	12	18	12	18	12	18	12	18
K1000 cl2	3.2	6.7	7.0	61.2	69.2	3.7	2.9	13.8	18.6
K1000 cl3	3.1	6.8	7.0	67.2	76.9	4.5	2.3	15.6	21.0
84-16-4-1	2.9	5.2	5.8	41.5	50.6	3.2	1.8	5.5	7.8
84-16-4-7	3.4	5.9	6.1	46.3	56.8	2.4	1.5	7.2	10.9
84-17-4-1	2.9	5.4	6.3	43.0	53.5	2.0	1.5	6.9	10.0
85-11-1-1	2.5	5.0	5.3	39.1	43.8	2.5	2.2	4.1	5.7
85-11-1-2	2.9	5.5	5.9	38.3	44.4	3.0	1.5	4.2	6.3
85-15-1-11	3.1	5.1	5.1	31.7	37.9	2.5	1.7	3.7	3.9
K156 x K376	3.6	6.2	6.2	63.9	72.0	3.0	2.3	14.2	19.5
KX3 cl1	3.2	6.4	6.8	57.3	69.3	2.1	1.6	11.7	18.4
KX3 cl2	3.9	6.7	6.8	55.8	58.3	1.7	1.2	11.0	12.2
KX2 cl2	3.6	6.5	7.1	58.2	65.5	2.9	1.7	12.2	17.3
K636	4.5	6.5	7.0	49.4	54.7	1.7	1.5	9.0	11.6
KX2 (seeds)	2.6	4.8	4.9	47.7	60.5	3.2	2.2	7.6	11.5
Average	3.2***	5.9***	6.2***	50.5***	58.1***	2.8 ***	1.8***	9.1***	12.5***
LSD $_{P=0.5}$	0.5	0.7	0.6	9.8	11	0.75	0.57	3.6	5.8

***: Significance at $p<0.001$.

The heights of entries ranged from 2.6 to 4.5 m, 4.8 to 6.8 m, and 4.9 to 7.1 m at the age of 6, 12, and 18 months respectively. At the age of 6 months, entries with the greatest heights were K636 (*L. leucocephala*), K156 x K376 (*L. diversifolia* x *L. pallida*), and KX2 cl2. K1000 clones did not show an advantage of growth at that age. At the ages of 12 and 18 months K1000 clones, along with K636 (*L. leucocephala*), KX3 cl1 (*L. diversifolia* x *L. pallida*), and KX2 cl2, outgrew all other entries in terms of height. However, K1000 clones did not significantly outgrow those top hybrids, and only outgrew one of the two controls, KX2 (seeds).

The DBHs of the entries ranged from 31.7 to 67.2 mm and 37.9 to 76.9 mm at the age of 12 and 18 months respectively. Hybrids had the largest DBHs were K1000 cl3, K1000 cl2, KX3 cl1, and K156 x K376 (*L. diversifolia* x *L. pallida*) at both ages (Table 3.2). The DBH of K1000 cl3 reached 76.9 mm after 18 months following planting, 32% greater than the overall mean of the trial. It was also significantly larger than the DBHs of two controls, K636 and KX2 (seeds). The DBH of K1000 cl2, however, was significantly larger than that of control KX2 (seeds), but not K636. Other triploid clones from different crosses had significantly smaller DBHs than K1000 clones.

All the entries produced multiple stems, and the overall mean of the number of main stems of the entries was 2.8 to 1.8 at the age of 12 and 18 months respectively. The number of main stems ranged widely from 1.7 to 4.5 at the age of 12 months, and 1.5 to 2.9 at the age of 18 months. K1000 clones had averages of 2.9 and 2.3 main stems after 18 months from planting, and they tended to have more main stems than other hybrids as well as the two controls.

The overall mean of wood volume of the entries was 9.1 and 12.5 dm^3 at the age of 12 and 18 months respectively. K1000 clones were among the entries producing the greatest wood volumes. K1000 cl3 and cl2 produced 21 and 18.6 dm^3 of wood respectively after 18 months of planting. Tetraploid hybrids KX3 cl1, KX3 cl2, and K156 x K376 (*L. diversifolia* x *L. pallida*) also produced high wood volumes, comparable to those of K1000 clones. On the other hand, those diploid hybrids and triploid hybrids other than K1000s, as well as the two controls were less productive on wood volume.

ANOVAs on height, DBH, the number of main stems, and wood volume are presented in Table 3.3. Highly significant ($P<0.0001$) differences of four traits were found among the entries at all three ages. Significant differences of height were also found among the replications at the ages of 6 and 18 months, and the interaction of entry and replication was significant at the ages of 12 and 18 months as well. In contrast to what was found in heights, no significant differences of DBH, the number of main stems, and wood volume were found among the replications. However, for all four traits, the interaction of entry and replication was highly significant. The highly significant interaction of entry and replication indicated the entries responded to three replications differently. This might be caused by wind and border effect. Trees facing main trade wind obviously grew worse than those inside the field, and trees on a border grew better than trees not on the borders.

Table 3.3. ANOVA on tree height, DBH, number of main stems, and wood volume based on single-tree data, using type III sum squares after 18 months growth at Waimanalo, Oahu (SET 01-4).

(a) Height	Growth period (months)					
	6		12		18	
Source	DF	Significance	DF	Significance	DF	Significance
Rep	2	*	2	NS	2	*
Entry (E)	13	***	13	***	13	***
E*rep	26	NS	26	*	26	***
Error	165		156		148	
CV (%)		20.4		14.5		12.5

(b) DBH	Growth period (months)			
	12		18	
Source	DF	Significance	DF	Significance
Rep	2	NS	2	NS
Entry (E)	13	***	13	***
E*rep	26	***	26	**
Error	166		152	
CV (%)		24.9		24.3

(c) Number of main stems	Growth period (months)			
	12		18	
Source	DF	Significance	DF	Significance
Rep	2	*	2	NS
Entry (E)	13	***	13	***
E*rep	26	***	26	NS
Error	166		160	
CV (%)		34.4		40.4

(d) Wood volume	Growth period (months)			
	12		18	
Source	DF	Significance	DF	Significance
Rep	2	NS	2	NS
Entry (E)	13	***	13	***
E*rep	26	***	26	***
Error	156		145	
CV (%)		34.4		40.4

*, **, and ***: Significantly different at $p<0.05$, 0.01, and 0.001, respectively.
NS: No significant difference.

The correlation coefficients of three traits (height, DBH, and wood volume) at three ages are indicated in Table 3.4. Correlations between 6-month height and all traits were relatively weak. However, the correlations among 12-and 18-month height, DBH, and wood volume were high. Especially, the correlation coefficients between 12-month DBH and 12-month wood volume, 12-month DBH and 18-month wood volume, 18-month DBH and 12-month wood volume, 18-month DBH and 18-month wood volume were very high ($r=0.98$, $p<0.0001$; 0.97, $p< 0.0001$; 0.96, $p<0.0001$; and 0.99, $p<0.0001$, respectively).

Table 3.4. Correlation coefficients of three traits at three ages from the trial of Waimanalo.*

	Ht.6†	Ht.12	Ht.18	DBH12	DBH18	Wd. vol.12	Wd. vol.18
Ht.6	1.00	0.69 (0.0062)	0.64 (0.0141)	0.38 (0.1847)	0.24 (0.4069)	0.41 (0.1494)	0.30 (0.2897)
ht.12		1.00	0.95 (<.0001)	0.82 (0.0004)	0.71 (0.0046)	0.84 (0.0002)	0.77 (0.0012)
Ht.18			1.00	0.73 (0.0030)	0.65 (0.0127)	0.75 (0.0020)	0.71 (0.0042)
DBH12				1.00	0.97 (<.0001)	0.99 (<.0001)	0.98 (<.0001)
DBH18					1.00	0.96 (<.0001)	0.99 (<.0001)
Wd.vol.12						1.00	0.98 (<.0001)
Wd.vol.18							1.00

*: Figures in parentheses are significance.
†: Ht.6, Ht.12, and Ht.18 represent height at age 6, 12 and 18 months respectively. DBH12 and DBH18 represent DBH at age 12 and 18 respectively. Wd.vol.12 and Wd.vol.18 represent wood volume at age 12 and 18 respectively.

SET 01-3, Hamakua, Hawaii Island

Overall survival rates in the Hamakua trial at the ages of 15 and 21 months were 83.6% and 78.6% respectively, and the survival rates of the entries ranged from 70 to 100%, and 60 to 100 % at two ages respectively. The survival rates were lower than those of Waimanalo. It was observed that plants on one side of the field, about first eight plots of all three replications, grew very poorly, with high mortalities and slow growth. The soil of that side looked normal except for one shallow ditch (~20 cm deep) passing through that area. The cause of poor growth in that area was unknown. No animal damage was found in this trial, and the psyllid damage was minor.

Mean height, DBH, wood volume, and number of main stems of the entries at the age of 15 and 21 months with LSD at p=0.05 level are indicated in Table 3.5.

The overall mean of the heights of the trial was 2.5 and 3.9 m at the age of 15 and 21 months respectively, notably smaller than at Waimanalo (5.9 and 6.2 m at the age of 12 and 18 months respectively). Mean heights of the entries ranged widely from 1.2 to 4.9 m at the age of 15 months and 1.3 to 6.7 m at the age of 21 months. Entries with the greatest heights were KX3 cl1 (*L. leucocephala* x *L. diversifolia*) (4.9 and 6.7 m at the age of 15 and 21 months, respectively), KX cl2 (3.8 and 5.8 m at the age of 15 and 21 months respectively), and K156 x K376 (*L. diversifolia* x *L. pallida*) (3.9 and 5.5 m at the age of 15 and 21 months respectively). Notably, those hybrids were also among those with the greatest heights at Waimanalo.

Table 3.5. Average tree height, diameter at breast height (DBH), wood volume, and number of main stems after 21months growth of *Leucaena* hybrids at Hamakua, Hawaii Island (SET 01-3).

		Height (m) at age (months) of		DBH (mm) at age (months)		Wood volume (dm^3) at age (months) of		# of main stems at age (months) of
	Entries	15	21	15	21	15	21	21
Replicated	K1000 cl2	2.2	3.6	19.4	32.5	0.8	3.4	1.8
	K1000 cl3	1.7	2.5	26.6	23.1	2.7	2.0	1.6
	K1000 wcl1	1.8	2.6	19.3	29.9	0.6	2.2	2.6
	K1000 wcl10	3.7	5.2	33.6	42.6	2.4	5.8	2.0
	K1000 wcl2	2.1	3.5	15.7	30.7	0.4	1.9	1.7
	K1000 wcl6	1.6	2.5	12.4	21.3	0.3	1.1	1.8
	K1000 wcl7	2.0	4.0	18.1	43.0	0.7	5.1	2.8
	84-15-1-11	3.0	4.4	23.2	41.5	1.1	4.2	2.4
	84-16-4-1	2.9	4.9	35.0	60.0	2.4	10.6	3.1
	84-16-4-7	3.0	4.5	42.5	60.0	4.1	13.2	2.4
	85-11-1-1	1.7	2.0	8.9	18.0	0.1	0.5	2.4
	85-11-1-2	2.1	2.5	14.1	21.6	0.3	0.8	2.7
	85-7-1-1	2.5	3.8	21.7	34.4	1.2	3.4	2.5
	85-9-3-8	2.3	3.0	15.2	28.7	0.5	1.8	2.2
	KX3 cl1	4.9	6.7	46.9	69.5	5.5	17.8	2.8
	KX3 cl2	2.7	4.2	22.7	34.9	1.3	2.9	1.8
	K156 x K376	3.9	5.5	50.6	83.7	5.7	23.2	3.1
	KX2 cl2	3.8	5.8	36.7	47.6	3.8	8.8	1.9
	K636	1.2	1.3	5.5	9.4	0.0	0.1	1.0
	KX2 (seeds)	1.6	2.3	14.4	27.5	0.5	2.4	1.6
Mean		2.5***	3.7***	22.1***	35.2***	1.7***	5.6***	2.2**
$LSD_{p=0.05}$		0.9	0.8	9.5	15.5	1.3	1.0	1.0
Augmented	K1000 wcl8	1.3	1.9	15.8	14.2	0.3	0.4	1.0
	K1000 wcl9	2.4	3.2	9.9	25.6	0.2	1.4	1.7
	K1000 wcl3	2.5	4.2	25.3	50.0	1.7	7.9	3.5
	K1000 wcl5	1.6	2.3	10.6	19.3	0.2	0.4	1.9
	84-17-4-1	3.6	5.4	37.2	60.2	3.1	8.7	2.6
	85-9-3-9	1.4	1.6	8.7	10.9	0.1	0.2	1.9
	85-7-1-8	1.1	2.4	7.5	28.1	0.0	0.8	2.5
	KX2 cl1	1.0	1.9	-	17.8	-	0.2	2.0

Most K1000 clones had only moderate heights, which varied widely among the clones. Only one clone of K1000--wcl10, as an exception, had the heights of 3.7 and 5.2 m at two ages, which were more or less comparable to those of the top hybrids. Two K1000 clones, cl2 and cl3, which grew extremely fast with a height of 7.0 m at the age of 18 months at Waimanalo, had only the heights of 3.6 and 2.5 m at Hamakua at the age of 21 months.

The controls, K636 and KX2 (seeds) had very low heights (1.3 and 2.3 m after 21 months of planting), seemingly having bad adaptation to this high-elevation site.

The DBHs of the entries varied widely from 5.5 to 46.9 mm, and 9.4 to 83.7 mm at the age of 15 and 21 months respectively. The entries with the largest DBHs were K156 x K376 (*L. diversifolia* x *L. pallida*) and KX3 cl1 (*L. leucocephala* x *L. diversifolia*). Their DBHs reached 50.6 and 83.7 mm, 46.9 and 69.5 mm at the age of 15 and 21 months respectively. Two clones of hybrid *L. pulverulenta* x *L. diversifolia*, 84-16-4-1 and 84-17-4-1 also had a large DBH of 60.0 mm at the age of 21 months. K1000 clones, however, had only moderate DBHs ranging from 21.3 to 43 mm at the age of 21 months. The variation of DBH among K1000 clones was obvious. K1000 wcl7 and K1000 wcl10 had larger DBHs than all other clones. K1000 cl2 and cl3, among hybrids with the largest DBHs at Waimanalo, ranked very low at Hamakua.

The overall mean of wood volume of the trial was 1.72 and 5.6 dm^3 at the age of 15 and 21 months respectively (Table 3.5). Wood volumes of the entries also varied remarkably. At the age of 21 months, the wood volume of hybrids K156 x K376 (*L. diversifolia* x *L. pallida*) and KX3 cl1 (*L. leucocephala* x *L. diversifolia*) was as high as 23.23 and 17.83 dm^3 respectively. Entries with moderate wood volumes were found on

L. pulverulenta x *L. diversifolia* (84-16-4-1 and 84-16-4-6). The wood volumes of all K1000 clones ranked very low among all the entries. Most replicated entries outgrew the control entries, K636 and KX2 (seeds) in wood volume.

The number of main stems of the entries was highly variable among the entries, ranging from 1.0 to 3.1 at the age of 21 months with an overall mean of 2.2 (Table 3.5). Hybrid K156 x K376 (*L. diversifolia* x *L. pallida*) had the highest number of main stems. Most K1000 clones were as branchy as other hybrids.

ANOVAs on height, DBH, wood volume, and the number of main stems based on single-tree data at two ages are presented in Table 3.6. Highly significant ($p<0.001$) differences of all four traits were found among the entries at two ages. Significant differences were also frequently found among the replications for many instances. As found in the Waimanalo trial, the entry by replication interaction for height was significant at both ages.

Table 3.6. ANOVA on tree height, DBH, number of main stems, and wood volume based on single-tree data, using type III sum squares after 21 months growth at Hamakua, Hawaii (SET 01-3).

	Height at the age of				DBH at the age of				Wood volume at the age of				# of main stems at age	
	15 ms.		21 ms.		15 ms.		21 ms.		15 ms.		21 ms.		21 ms.	
Source	DF	SG	DF	SG	DF	SG	DF	SG	DF	SG	DF	SG	DF	SG
Rep	2	NS	2	**	2	*	2	***	2	**	2	*	2	NS
Entry (E)	19	***	19	***	19	***	19	***	19	***	19	***	19	***
E*rep	38	***	38	**	37	*	37	NS	37	NS	37	NS	37	NS
Error	199		183		143		168		143		168		167	
CV(%)		34.4		23.2		51		49.6		69.8		20.3		47.7

SG: Significance.
*, **, and ***: Significantly different at $p<0.05$, 0.01, and 0.001, respectively.
NS: No significant difference.

The correlation coefficients of three traits (height, DBH, and wood volume) at two ages are shown in Table 3.7. The correlations among height, DBH, and wood volume were generally high. Especially, the correlation coefficients between 15-month DBH and 15-month wood volume, 21-month DBH and 21-month wood volume, were very high ($r=0.97$, $p<0.0001$; 0.96, $p< 0.0001$, respectively.).

Table 3.7. Correlation coefficients of three traits at two ages from the trial of Hamakua.*

	DBH18	DBH21	Height18	Height21	W.Vol.18	W.Vol.21
DBH18	1.00	0.93 (<.0001)	0.89 (<.0001)	0.76 (<0.0001)	0.97 (<.0001)	0.92 (<.0001)
DBH21		1.00	0.84 (<.0001)	0.79 (<.0001)	0.87 (<.0001)	0.96 (<.0001)
Height18			1.00	0.85 (<.0001)	0.84 (<.0001)	0.81 (<.0001)
Height21				1.00	0.68 (0.0009)	0.72 (0.0004)
W.Vol.18					1.00	0.92 (<.0001)
W.Vol.21						1.00

*Figures in parentheses are significance.

SET 01-2, Kona, Hawaii

The DBH, height, and wood volume of three entries in the Kona trial at the age of 20 months are presented in Table 3.8. K1000 cl3 showed much greater growth rates than the two control entries. The mean DBH, height, and wood volume of K1000 cl3 reached 81.5 mm, 9.4 m, and 31.9 dm^3 respectively. The mean DBH, height, and wood volume of K1000 cl3 were almost three, twice, and 10 times of those of the two controls.

Table 3. 8. Average tree height, DBH, and wood volume after 20 months growth of 3 *Leucaena* entries at Kona, Hawaii Island.

	DBH (mm)	Height (m)	Wood volume (dm^3)
K1000 cl3	81.5	9.4	31.9
K 636	27.3	4.5	3.11
KX2 (seeds)	27.3	4.2	2.29

Comparison of growth at Waimanalo and Hamakua

The comparison of growth of 13 entries planted both at Waimanalo and Hamakua in two similar periods is summarized in Table 3.9. The growth of entries at Hamakua varied more widely than that at Waimanalo. For example, the DBHs of the entries at Hamakua ranged from 9.4 to 83.7 mm, while they ranged only from 37.9 to 76.9 mm at Waimanalo. The different performance of the same entry in two sites was manifest. Two K1000 clones grew extremely well at Waimanalo in terms of height, DBH, and wood volume; however, they grew poorly at Hamakua. The opposite example is the clones of *L. pulverulenta* x *L. diversifolia* (84-16-4-1 and 84-16-4-7), which tended to grow better at Hamakua than at Waimanalo. Two tetraploid species, K156 x K376 (*L. diversifolia* x *L. pallida*) and KX3 cl1 (*L. diversifolia* x *L. leucocephala*), however, grew extremely well in both sites. All the results clearly indicate that there existed the interaction of entries by sites.

Table 3.9. Comparison of the growth of entries at Waimanalo and Hamakua in two similar growth periods (21 months at Hamakua, and 18 months at Waimanalo).

	Height (m)		DBH (mm)		Wood volume (dm^3)		# of main stems	
Entries	Hamakua	Waimanalo	Hamakua	Waimanalo	Hamakua	Waimanalo	Hamakua	Waimanalo
K1000 cl2	3.6	7.0	32.5	69.2	3.4	18.6	1.8	2.9
K1000 cl3	2.5	7.0	23.1	76.9	2.0	21.0	1.6	2.3
84-16-4-1	4.9	5.8	60.0	50.6	10.6	7.8	3.1	1.8
84-16-4-7	4.5	6.1	60.0	56.8	13.2	10.9	2.4	1.5
85-11-1-1	2.0	5.3	18.0	43.8	0.5	5.7	2.4	2.2
85-11-1-2	2.5	5.9	21.6	44.4	0.8	6.3	2.7	1.5
84-15-1-11	4.4	5.1	41.5	37.9	4.2	3.9	2.4	1.7
K156 x K376	5.5	6.2	83.7	72.0	23.2	19.5	3.1	2.3
KX3 cl1	6.7	6.8	69.5	69.3	17.8	18.4	2.8	1.6
KX3 cl2	4.2	6.8	34.9	58.3	2.9	12.2	1.8	1.2
KX2 cl2	5.8	7.1	47.6	65.5	8.8	17.3	1.9	1.7
K636	1.3	7.0	9.4	54.7	0.1	11.6	1.0	1.5
KX2 (seeds)	2.3	4.9	27.5	60.5	2.4	11.5	1.6	2.2

Discussion

This is the first study evaluating the performance of *Leucaena* hybrids. Seedless triploid hybrid K1000 grew rapidly in warm areas such as Kona and Waimanalo, where it outgrew the most popular *L. leucocephala* accession K636 and advanced generations of KX2 in terms of DHB, height, and wood volume. K1000 clones did not significantly outgrow those fast-growing tetraploid hybrids such as KX2 cl2, KX3 cl1 (*L. leucocephala* x *L. diversifolia*), and K156 x K376 (*L. diversifolia* x *L. pallida*) at Waimanalo, but their growth ranked above all the entries.

In cool areas like Hamakua of Hawaii Island, the growth of most K1000 clones was greatly stunted. However, some tetraploid hybrids, such as KX3 cl1 (*L. leucocephala* x *L. diversifolia*) and K156 x K376 (*L. diversifolia* x *L. pallida*), grew extremely fast. Both *L. diversifolia* and *L. pallida* are highland species with moderate tolerance to cold temperatures and are frequently used to breed cold tolerant hybrids (Brewbaker and Sorensson, 1994; Hughes, 1998). Some non-K1000 triploid hybrids, such as the clones

of *L. pulverulenta* x *L. diversifolia*, also had moderate growth rates at Hamakua. Meanwhile, due to high variation of growth among the clones of K1000, some K1000 clone like wcl7 also had relatively high growth rates. Therefore, there are still opportunities to plant fast-growing seedless hybrids in cool areas.

The mean annual temperature of Hamakua station (17 °C) is about 8 °C lower than that of Waimanalo station (25 °C), and Hamakua station receives more rainfall than Waimanalo station. The slow growth of K1000 clones at Hamakua station was seemingly caused by the typically low temperatures, and may suggest a limitation of K1000 clones. Caution should be taken before their expansion to cool areas.

K1000 trees tended to form multiple stems in all three trials, as many other hybrids did, in a dense planting spacing (2 x 1.5 m). Early pruning might be needed if a single bole is preferred in wood production. The characters of fast growth and seedlessness make the K1000 clones very promising in hardwood production and environmental conservation in certain areas. Moreover, successful development of vegetative propagation method for K1000 clones (see chapter 2) makes it possible to expand these hybrids to a large area at a reasonable cost.

Certain problems should be solved before the expansion of K1000 plantation. At Waimanalo, K1000 trees, along with other hybrids propagated by cuttings, encountered over-blow in stormy winter. In contrast, no trees raised from seeds were blown over by wind. The lodging was apparently due to the lack of taproot of the hybrids. There are some possible remedies for this problem. One is to induce the formation of taproots in propagules by using long dibble tubes instead of square pots before planting. Also proper planting time is surely important for the development of root system. Trees planted in

early summer should have longer time to develop strong root system than those planted later, and they have more chance to stand in stormy weather.

Our results are based on limited test sites in short time. More studies are needed to fully understand the growth of K1000 and other useful *Leucaena* hybrids for wood production. Subsequent studies should be focused on the wood qualities of *Leucaena* hybrids to justify their use as high-value hardwoods.

Conclusions

K1000 clones grew fast in warm areas such as Waimanalo and Kona in terms of height, DBH, and wood volume, where it outgrew the two control entries (seed derived) and had growth rates comparable to other top hybrids. Most K1000 clones did not grow well in cool area of Hamakua, but variations in growth rate among K1000 clones were observed there. Clones with relatively higher growth rates might be selected for future plantations. Tetraploid hybrids KX3 (*L. leucocephala* x *L. diversifolia*) and *L. diversifolia* x *L. pallida* grew fastest both at Waimanalo and Hamakua. Some clones of triploid hybrid *L. pulverulenta* x *L. diversifolia* also grew relatively well at Hamakua and therefore are potential seedless hybrids for that area. K1000 clones tended to produce multiple stems, as did most hybrids. Thus early pruning is possibly needed for a single straight bole. At Waimanalo, clonal plants from cuttings were blown over in stormy winter, apparently due to the lack of taproots. Thus, the induction of taproots is needed for clonal plants to stand in stormy weather.

CHAPTER 4. TETRAPLOID INDUCTION IN *LEUCAENA* SPECIES

Introduction

There are 22 species in the genus *Leucaena,* among which *L. leucocephala* is the most important and widely used species around the world. *Leucaena leucocephala* is a very attractive species for many uses, such as animal fodder, fuelwood, biomass, wood production, alley crop, and environmental conservation. Despite its adaptability to a wide range of environments, this species does not adapt to acid soils, cold weather, and water-logged soils. The burst of psyllids two decades ago, an insect that damages young leaves and shoots of *L. leucocephala*, severely impacted the planting of *L. leucocephala*. The evidently narrow genetic base of *L. leucocephala* makes it difficult to overcome these limitations within the species, since alternate genotypes of the species with resistance or tolerance to adverse environments are not readily available.

Two uses of induced tetraploids are of potential importance. The main commercial species, *L. leucocephala*, is a tetraploid, and gene introgression from other tetraploids offers attractive opportunities for genetic advance. Induced tetraploids also expand the species for creating seedless triploids in this genus of 17 diploid and 5 tetraploid species.

Researchers have proposed using lesser-known species for genetic improvement of *L. leucocephala*. Lesser-known species have a number of desirable features that *L. leucocephala* lacks (Hughes, 1998). These features include high resistance to psyllids (e.g., *L. pallida*, *L. trichandra*, *L. collinsii*, and *L. esculenta*), tolerance to acid soils (e.g., *L. diversifolia*, *L. esculenta*, and *L. shannonii*), tolerance to cold temperatures (e.g., *L.*

retusa, *L. greggii*, *L. pallida*, *L. pulverulenta*, and *L. diversifolia*), good wood qualities (e.g., *L. collinsii*, *L. shannonii*, and *L. salvadorensis*), and low minosine contents (e.g., *L. pulverulenta*, *L. esculenta*, and *L. diversifolia*). Efforts by Sorensson (1987, 1993), Pan (1985), and other researchers produced more than one hundred interspecific *Leucaena* hybrids, and some of them are very promising for forage and wood production (Brewbaker and Sorensson, 1990; Sorensson, 1993).

There are problems associated with using lesser-known species in *Leucaena* breeding programs. Most of *Leucaena* species are diploid (2n=52 and 56), while the most important species like *L. leucocephala*, *L. diversifolia*, and *L. pallida* are tetraploid (2n=104). Generally, genes flow freely among *Leucaena* species due to high crossability among the species (Sorensson, 1993). However, most crosses between tetraploids and diploids produce sterile or unstable triploids, which hinder the production of further breeding generations (Sorensson, 1997). The triploids might be rather promising, but the direct use in breeding will be prohibited due to sterility or unstable offspring (Sorensson, 1997). One such example is the triploid hybrid *L. trichandra* x *L. leucocephala*. Sorensson (1997) found that this triploid is very attractive with high leaf yields and good resistance to psyllids, but its offspring are low in vigor. Some triploid hybrids readily produce stable progenies after the F1 generation, but it is possible for progenies to lose traits of economic interest (Sorensson, 1997).

There is difficulty of transferring genes from some diploid species to target tetraploid species. One such example is *L. collinsii*. The triploid of *L. collinsii* and *L. leucocephala* has poor seedling vigor and high field mortality (Sorensson and Brewbaker, 1987). To overcome the problems of using lesser-known diploid species, Sorensson and

Brewbaker proposed using unreduced gametes of diploid species to produce fertile tetraploid hybrids instead of triploids (Sorensson and Brewbaker, 1987; Sorensson, 1997). Unreduced gametes are commonly found in *Leucaena* species, both in tetraploid and diploid species (Sorensson and Brewbaker, 1987; Schifino-Wittmann et al., 1997). However, the frequencies of unreduced gametes are usually low. Sorensson and Brewbaker reported 0 to 0.1% unreduced pollen grains in most accessions that they examined. However, they found greater than 1% unreduced pollen grains in some accessions (Sorensson and Brewbaker, 1987). Schifino-Wittmann and Simioni (1999) reported that higher percentages of unreduced pollen occur in some accessions of *L. leucocephala* subsp. *glabrata* (7.0%), *L. pulverulenta* (7.5%), and *L. trichandra* (up to 12%). They suggest that the frequency of unreduced gametes in *Leucaena* is controlled genetically (Schifino-Wittmann and Simioni, 1998; 1999).

No effective breeding use of unreduced gametes has been reported in *Leucaena*, although the possibility was validated by Sorensson and Brewbaker (1987). A vigorous hybrid of diploid *L. retusa* and tetraploid *L. pallida* was confirmed to be a tetraploid with 2n=108. The hybrid apparently resulted from the fusion of an unreduced egg of *L. retusa* with a normal pollen grain of *L. pallida.* Sorensson (1997) proposed a breeding strategy to use unreduced gametes to produce tetraploid hybrids. The method utilized clones of self-incompatible diploid species inter-planted with tetraploid species like *L. leucocephala* in an isolated place. Seed-bearing tetraploid hybrids would then be sought among the more numerous sterile triploid progeny.

It is obvious that using unreduced pollen grains to produce tetraploids might be very ineffective, given no special procedures to increase their frequency and to separate

them from normal pollen grains. Moreover, no such procedures have been reported in *Leucaena*. Physical (high temperatures) and chemical (colchicine) treatments have been effectively used in *Populus* spp. to produce unreduced gametes at high frequencies (pollen and eggs) in China (Kang, 1996; Li, 1997). These researchers also separated unreduced pollen from normal pollen successfully by using a sieve, thus dramatically increasing the frequency of unreduced pollen.

Induction of autotetraploidy in diploid *Leucaena* species might be more straightforward than the use of unreduced gametes. Chromosome doubling is very common in plant breeding. New polyploids may be useful in breeding programs, and they may also be of high value for direct uses.

Pan (unpublished) did a preliminary study of somatic chromosome doubling in *Leucaena*. He treated diploid seeds with aqueous colchicine solutions, and the effects of induction were determined by morphological abnormalities of seedlings from treated seeds.

This study focused on induction of tetraploids from somatic cells of *Leucaena* diploid species. The aims of the study were:

1) to develop a simple and reliable method to induce tetraploid plants;
2) to develop a method to readily identify chromosome-doubled plants;
3) to investigate the morphological characteristics of induced tetraploids.

Materials and methods

Somatic chromosome doubling

Five diploid species, including *L. esculenta*, *L. collinsii, L. trichandra*, *L. lanceolata*, and *L. pulverulenta* were involved in the study. Accessions used in the study are summarized in Table 4.1.

Table 4.1. *Leucaena* accessions used in tetraploid induction.

Species	Accession number	Chromosome number (2n)	Origin
L. collinsii	K911	56	Zacapa, Guatemala
	K912	56	Zacapa, Guatemala
L. esculenta	K1057	56	Oaxaca, Mexico
L. lanceolata	K468	52	Chiapas, Mexico
	K952	52	Chiapas, Mexico
L. pulverulenta	K957	56	Tamaulipas, Mexico
L. trichandra	K909	52	Guatemala City, Guatemala
	K919	52	Guatemala City, Guatemala

Leucaena seeds were first nicked on the cotyledon side of the seeds to facilitate water absorption. They were then sown in sterile medium in 6-inch pots in the greenhouse. They were grown under standard culture management. Most seeds germinated in 5 days. After the two cotyledons of the seed emerged from the medium and had stretched horizontally, a small piece of cotton saturated with 0.1% aqueous colchicine (Sigma) solution was placed on the seedling meristems. Each pot was then covered with a sheet of clear plastic to prevent desiccation of the cotton balls, and placed under 30% shade to minimize heat accumulation. After one or two days the cotton balls were removed, and the shoots were washed with water. Cotton balls dipped in water were used as the control. After colchicine or water treatments, the plants were moved to

benches under full sun. The numbers of surviving plants and shoots were counted after one month.

When stems of treated seedlings had a total of 4 to 5 nodes, the tips of shoots were cut off for rooting according to the procedure outlined in Chapter 2. After the shoots rooted, at least three root tips from different positions of the stem were collected for chromosome counts. For chromosome counts, root tips were then pretreated with 300 ppm cycloheximide solution for 5 to 6 hours. The root tips were fixed in Carnoy's fixative for at least 0.5 hour. The root tips were washed in water and softened in a solution of 1:1 95% ethanol and concentrated hydrochloric acid for 5 to10 minutes. The root tips were washed again, and the excised meristem of the root tip was squashed in a drop of carbol-fuchsin stain. Prepared slides were observed under a Carl Zeiss microscope, and pictures were taken with a Nikon Coolpix 4500 digital camera. The camera was connected to the microscope with an eyepiece (Periplan 10 x /18, Leitz Wetzlar, Germany).

All apparently tetraploid plants were subjected to at least one more chromosome inspection. Tetraploid induction rates were calculated as the ratio (%) of induced tetraploids to the number of treated plants. Some induced tetraploid plants were chimeras of tetraploid and diploid, which were counted as 0.5 instead of 1.

Morphological and biological characteristics of induced tetraploids

Leaflet thickness, leaflet size, and the size of flower heads

Longitudinal and transverse measurements of tetraploid and diploid leaflets were determined with a caliper. All leaflets were from plants at the same age to avoid

variation caused by different growth stages. At least 20 leaflets from each tetraploid and diploid randomly selected from different locations of plants were measured. For leaflet thickness, five leaflets were stacked, and the thickness of the stack was measured; then the thickness of a single leaf was calculated.

Self-fertility

Tetraploid and diploid plants were moved to the greenhouse in early morning (before 6:30 a.m.) to test for self-fertility by hand pollination. Flower heads shed pollen at about noon, and pollination was conducted by touching the stigmas with pollen collected on the flat handle of a pair of forceps from the same plant. Pollinated plants were moved out of the greenhouse after 3:00 p.m. The flower heads were labeled after pollination, and fertilization was determined on the basis of fruit set after 10 days. Pollination before and after the plants were moved to the greenhouse was expected to be rare, since no pollen is shed during those periods. Furthermore, experience indicates that stigmas were not receptive the day following anthesis.

Results

Effects of colchicine treatments on plant survival

The effects of 0.1% colchicine treatments on the survival of *Leucaena* seedlings are summarized in Table 4.2. In general, *Leucaena* seedlings were very tolerant of the toxicity of colchicine. Neither 24 nor 48-hour treatments caused high rates of seedling and apical death. The 24-hour treatment of 0.1% colchicine did not cause any seedling death in any species, but caused some dieback of apical points (0 to 86.7%). The 48-hour

treatments caused some death of seedlings (0 to 17.9%), and more dieback of apical points (9.1 to 95.5%). The controls showed no seedling or apical point death.

The death rates of seedlings and apical points after colchicine treatments appeared to differ widely among the species. *Leucaena collinsii* was more vulnerable to colchicine treatments than other species (Table 4.2). The death of apical shoots followed the induction of abnormalities in which the shoots became callus-like, with retarded growth (Figure 4.1). Axillary buds were often produced after the death of apical shoots. Early leaflet distortions and enlargements could also be observed (Figure 4.1).

Tetraploid induction

Most cuttings from treated seedlings rooted well, and roots from cuttings were suitable for making chromosome counts. The chromosomes of diploids are shown in Figure 4.2, and those of tetraploids are shown in Figure 4.3. It was usually difficult to determine the chromosome numbers in root tip cells precisely, but it was easy to tell induced tetraploid cells from diploid cells by approximate determination of chromosome numbers.

Tetraploid plants were found in all species after colchicine treatments (Table 4.3); no tetraploid plants were found in the controls. Tetraploid induction rates differed greatly among the species, although the sample size was too small to establish significance. In general, the 48-hour treatment had higher induction rates than the 24-hour treatment (6.7 to 30.5% vs. 0 to 21.9%). *Leucaena collinsii* and *L. esculenta* apparently had higher induction rates than other species.

Figure 4.1. Effects of 48-hour 0.1% colchicine treatment on seedlings of *Leucaena collinsii* K911. A: Control. B: Distorted leaflets. C: Callus-like swelling of an apical shoot. D: Regenerated axillary buds after the death of the apical shoot.

Table 4.2. Effects of 0.1% colchicine treatments on seedlings and apical buds of seedlings of *Leucaena*.

A. 24-hour treatment

Species	K No.	No. of treated seedlings	No. of dead seedlings	% of dead seedlings	No. of dead apical buds	% of dead apical buds
L. collinsii	K911	20	0	0	14	70.0
	K912	15	0	0	13	86.7
L. esculenta	K1057	24	0	0	0	0.0
L. lanceolata	K468	10	0	0	0	0.0
	K952	14	0	0	0	0.0
L. pulverulenta	K957	35	0	0	4	11.4
L. trichandra	K919	29	0	0	1	3.4

B. 48-hour treatment

Species	K No.	No. of treated seedlings	No. of dead seedlings	% of dead seedlings	No. of dead apical buds	% of dead apical buds
L. collinsii	K912	22	2	9.1	21	95.5
	K911	24	0	0.0	20	83.3
L. esculenta	K1057	28	5	17.9	6	21.4
L. lanceolata	K952	30	2	6.7	6	20.0
L. pulverulenta	K957	29	0	0.0	6	20.7
L. trichandra	K909	22	0	0.0	2	9.1

Table 4.3. Tetraploid induction rates from 0.1% colchicine treatments of *Leucaena* seedlings.

A. 24-hour treatment

Species	K No.	No. of plants examined	No. of tetraploids	No. of chimeras	% of induction
L. collinsii	K912	11	1	0	9.1
	K911	16	3	1	21.9
L. esculenta	K1057	13	1	1	11.5
L. lanceolata	K952	13	0	0	0.0
	K468	11	0	1	4.6
L. pulverulenta	K957	30	3	2	13.3
L. trichandra	K919	22	3	1	16.0

B. 48-hour treatment

Species	K No.	No. of plants examined	No. of tetraploids	No. of chimeras	% of induction
L. collinsii	K912	15	4	1	30.0
	K911	15	1	0	6.7
L. esculenta	K1057	8	3	0	37.5
L. lanceolata	K952	30	2	1	8.3
L. pulverulenta	K957	16	1	3	15.6
L. trichandra	K909	15	2	1	16.7

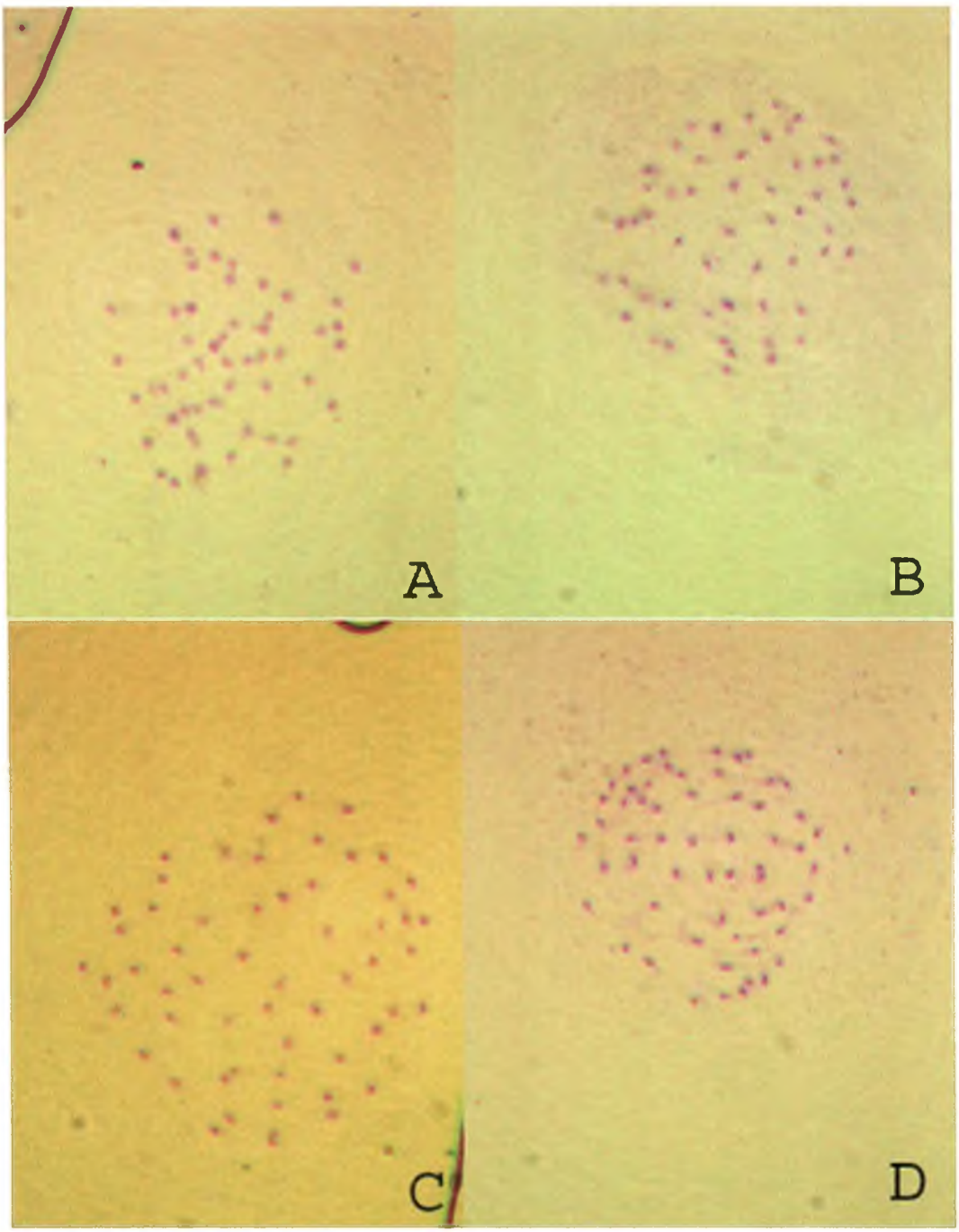

Figure 4.2. Chromosomes in root-tip cells of diploid *Leucaena* species. A: *L. lanceolata* K468, 2n=52. B: *L. pulverulenta* K957, 2n=56. C: *L. esculenta* K1057, 2n=56. D: *L. collinsii* K911, 2n=56.

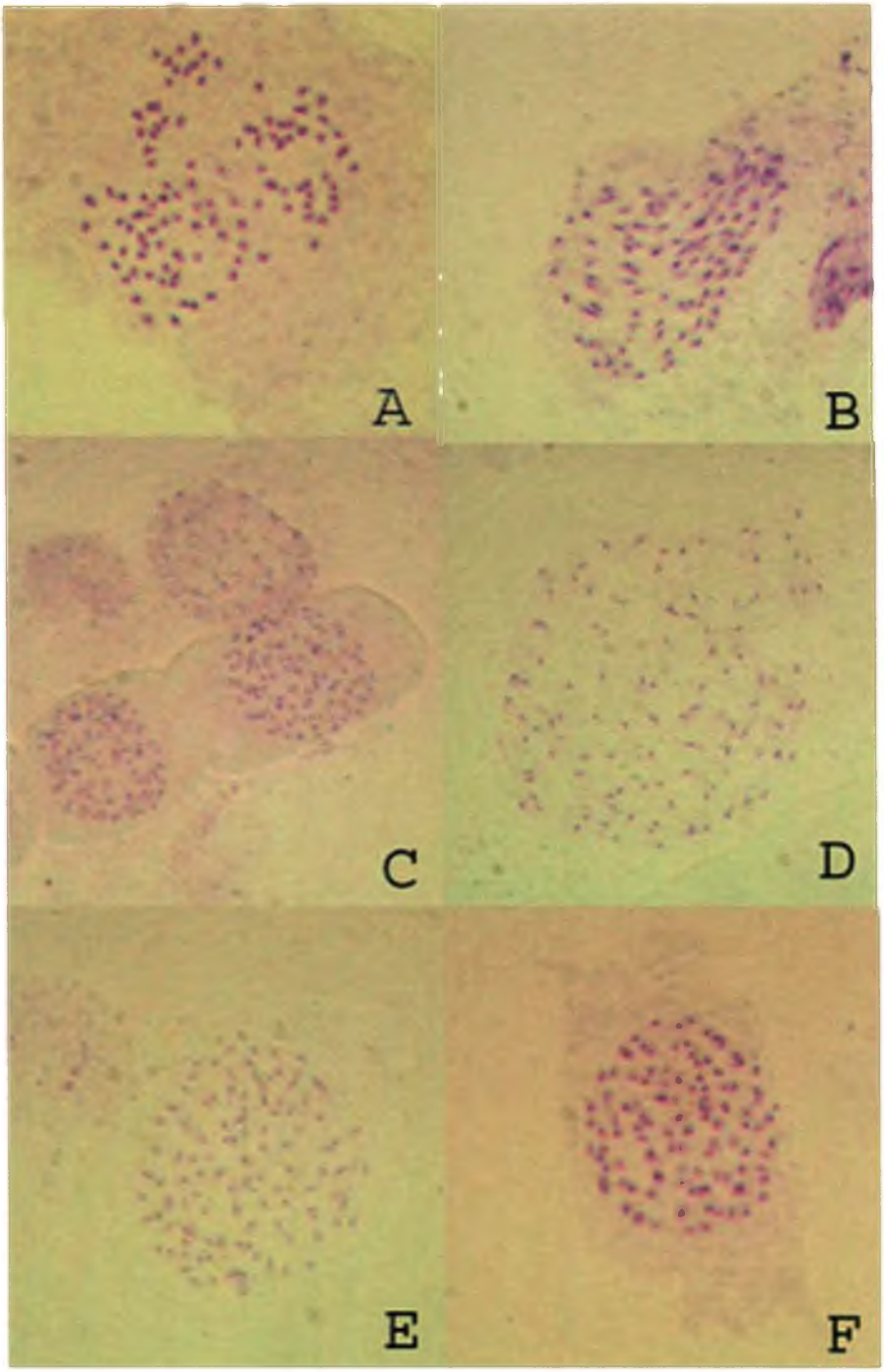

Figure 4.3. Chromosomes in root-tip cells of induced tetraploid *Leucaena*. A: *L. lanceolata* K952, 2n≈104. B: *L. collinsii* K911, 2n≈112. C: *L. trichandra* K919, 2n≈104. D: *L. collinsii* K912, 2n≈112. E: *L. lanceolata* K468, 2n≈104. F: *L. pulverulenta* K957, 2n≈112.

Some colchicine-induced plants were chimeras of diploid and tetraploid cells in different sections of the same layer of meristem. Some chimeras became pure diploid or tetraploid plants after subsequent cloning, while others remained chimeric for the duration of study.

Morphological and biological characteristics of induced tetraploids

Morphological modifications were observed in induced tetraploids in all species studied. The most conspicuous changes were in the size and thickness of leaflets, and in the size of flower heads.

Size and thickness of leaflets

Table 4.4 summarizes the sizes of leaflets of induced tetraploid species. *Leucaena collinsii* accessions K911 and K912 had larger and thicker leaflets compared to diploid counterparts. Leaflets of induced tetraploids of *L. lanceolata* K952 were thicker, but not larger, than those of diploids. On the other hand, the leaflets of induced tetraploid of *L. trichandra* K919 were neither larger nor thicker than those of diploids. Data are limited and conclusions only tentative.

Color of leaf

The leaf color of most induced tetraploids was darker than that of control diploids. There was no color difference between tetraploid *L. trichandra* K919 and its diploid counterparts.

Table 4.4. Morphological characteristics of induced tetraploids and diploids of *Leucaena*.

A. *L. collinsii* K911

	Length of leaflets (mm)	Width of leaflets (mm)	Thickness of leaflets (mm)	Diameter of flower heads (cm)
Check 1	6.8±0.68	2.1±0.22	0.12±0.016	
Check 2	6.4±0.34	1.7±0.06	0.12±0.009	1.6
Check 3	5.5±0.69	1.8±0.15	0.12±0.011	
Tetraploid	7.6±0.82	2.2±0.16	0.18±0.018	2.2

B. *L. collinsii* K912

	Length of leaflets (mm)	Width of leaflets (mm)	Thickness of leaflets (mm)
Check 1	8.87±0.69	2.15±0.17	0.08±0.007
Check 2	8.93±1.54	2.26±0.44	0.08±0.014
Check 3	8.63±0.93	2.31±0.29	0.07±0.011
Check 4	8.80±0.85	2.22±0.17	0.07±0.005
Tetraploid 1	9.93±1.11	2.65±0.29	0.15±0.013
Tetraploid 2	11.02±0.84	2.82±0.17	0.19±0.023
Tetraploid 3	11.33±0.67	3.03±0.17	0.12±0.010
Tetraploid 4	11.83±1.02	2.79±0.35	0.14±0.013

C. *L. lanceolata* K952

	Length of leaflets (mm)	Width of leaflets (mm)	Thickness of leaflets (mm)
Check1	44.6±6.3	31.9±4.3	0.14±0.009
Check2	34.8±2.9	21.0±1.8	0.13±0.009
Check3	50.7±9.7	37.1±6.1	0.16±0.011
Check4	52.1±5.0	31.8±4.3	0.15±0.012
Tetraploid	40.8±8.5	27.5±5.9	0.20±0.008

D. *L. trichandra* K919

	Length of leaflets (mm)	Width of leaflets (mm)	Thickness of leaflets (mm)
Check1	8.26±0.40	2.17±0.29	0.10±0.004
Check2	6.07±0.44	1.54±0.15	0.09±0.008
Check3	9.05±0.70	2.22±0.09	0.09±0.008
Tetraploid	9.03±0.62	2.69±0.27	0.10±0.008

Size of flower heads (inflorescences)

Only one induced tetraploid plant of *L. collinsii* K911 flowered during the study. Its flower heads were larger than those of the controls (Table 4.4).

Self-fertility

The only flowering tetraploid of *L. collinsii* K911 was highly self-fertile. Six self-pollinated flower heads all set fruits, while no flower heads of diploid controls set fruit after self-pollination. It was also noted that open-pollinated flower heads of the tetraploid produced fruits, as did those of diploids in the nursery. Almost all flowering heads of tetraploids set pods in different numbers, apparently as a result of bee pollination.

Discussion

Artificial induction of tetraploids of *Leucaena* species turned out to be highly feasible. Tetraploid plants were obtained in all experimental species after treatment with 0.1% colchicine for 24 or 48 hours. Neither of the two treatment durations was significantly superior. In some species the induction rates were rather high. *Leucaena esculenta* and *L. collinsii* produced more than 25% tetraploid plants after colchicine treatments, clearly indicating that this induction method is feasible and reliable for the genus. This has also been true in other legumes such as clover (Brewbaker, 1952). In future work the method can be readily applied to diploid accessions of specific interest to researchers. Since the mortalities of seedlings and apical buds after current colchicine

treatments were low, it seems likely either higher colchicine concentrations or longer treatment durations may be useful to increase tetraploid induction rates in *Leucaena*.

The verification of tetraploid plants must be done very cautiously, since some plants initially identified tetraploids may actually be chimeric. Therefore, repeated inspection is needed to make sure the plants are stable tetraploids. Cloning and sub-cloning improved recovery of tetraploids.

Morphological changes were observed in induced tetraploids in some species. The most common changes were increased thickness of leaflets and darker color of leaf. Thicker leaflets also caused a tougher leaf texture. Induced tetraploid plants tended to have larger leaflets, but because of highly variable leaflet sizes within the species, this was not always distinguishable. The changes of thickness and color of leaflets were more consistent, as they have been in other instances of polyploid legumes. In future work on chromosome doubling, the appearance of such features can be used for early screening of induced tetraploids.

The only induced tetraploid that flowered appeared to be highly self-fertile, confirming the phenomenon that polyploidization often converts self-sterile diploids to self-fertile tetraploids (reviewed by Brewbaker, 1953). This phenomenon is also evident in petunia and alsike clover, where the diploid species is highly self-sterile. Some tetraploid counterparts are self-fertile in the first breeding cycle, and self-fertile plants tend to dominate the population after several breeding cycles (Brewbaker, 1953).

In species with large chromosomes, autopolyploid plants are highly sterile due to the irregularity of chromosomal pairing during meiosis (Sybenga, 1992). Irregularities of chromosome association during meiosis can result in the formation of multivalents,

trivalents, and univalents during metaphase I (Sybenga, 1992). Unlike bivalents, the multivalents, trivalents, and univalents of homologous chromosomes usually fail to segregate equally into daughter cells at anaphase I, causing unbalanced chromosome numbers in pollen or eggs (Sybenga, 1992). Such pollen and eggs are usually not viable, and therefore the plants have reduced fertility. Chromosomally unbalanced pollen and eggs may lead to aneuploid offspring if the gametes produced are viable. High fertility of tetraploid *L. collinsii* K911 indicates that it produces substantial numbers of viable pollen and eggs. However, it is unclear if the pollen and eggs are euploid; further monitoring of the chromosome number of selfed progeny of K911 should provide a means to assess this issue.

Seed crops such as such as cereals, especially self-pollinated species like barley commonly exhibit meiotic irregularities as tetraploids and are inferior to their diploid counterparts in this respect (Friedt, 1986; Evans and Rahman, 1989). However, many tetraploids of forage crops, such as Russian wildrye (*Psathyrostachys juncea*), ryegrass (*Lolium multiflorum*), and perennial ryegrass (*Lolium perenne*), have substantial increases in forage yield, vigor, resistance to disease, and palatability (Dewey, 1979; Wang and Berdahi, 1990).

Successful induction of tetraploids in *Leucaena* species can play a role as a genetic bridge for transfer of genes among the species, as has been done in crested wheatgrass (Asay and Dewey, 1979). Gene transfers from promising diploids such as *L. collinsii*, *L. esculenta*, *L. trichandra*, and *L. pulverulenta* to *L. leucocephala* may be facilitated by induced tetraploids. Thus, fertile tetraploid hybrids can be obtained from

crosses with *L. leucocephala*. Crosses between diploid species and induced tertaploids may also produce more seedless triploids potentially useful for wood production.

Conclusions

Artificial induction of tetraploids with the treatment of 0.1% colchicine was accomplished in 5 diploid *Leucaena* species. Morphological modification of induced tetraploids included size, thickness, and color of leaflets. Increased size of flower heads and self-fertility was also noted in the one species that flowered during the study. The morphological features generally exhibited by tetraploids may be useful in early screening for autotetraploids among plants treated with mitotic poisons such as colchicine.

SECTION TWO

KOA RESEARCH

CHAPTER 5. LITERATURE REVIEW ON KOA

Koa (*Acacia koa* Gray) is regarded as one of the most prestigious timber species in Hawaii, best known for its exceptionally fine wood. Koa wood is extremely valuable owing to its unusually curly grain, rich colors (yellow to deep purple), and excellent working properties. It is a legend and symbol in Hawaiian culture. It has been widely used for crafts, instruments, furniture, and utensils, and was famous for building canoes and surfboards (Whitesell, 1990; Sun, 1996). Koa wood and its products are highly demanded and undersupplied due to limited, declining supply on present markets (year 2002). The price of koa lumber exceeds that of all other hardwoods.

Koa is not only culturally and economically important, it is also an important component of Hawaii's pristine ecosystem. It is a nitrogen-fixing species, which nurtures soils through its rhizobial root system and leaf littering. Koa forests have protected Hawaii's precious watersheds historically, and they are important habitats for many insects and endangered birds (Whitesell, 1990).

Koa was once extensively thriving on Hawaiian Islands. Due to land clearing, over-logging, animal grazing, fire, and various insects, the resource of koa has decreased dramatically. Coverage of koa forest shrunk from 455 to 41 thousand hectares from 1963 to 1991 (Nelson et al., 1963; Brewbaker et al., 1991). Stock volume of commercial koa dropped from 34.1 to 7.1 x 10^5 m^3 between 1960 to 1980 (Nelson et al., 1963; Metcalf et al., 1978). Not only the volume of koa stands has decreased, its genetic composition has also deteriorated. Few "superior" trees can be found in wild koa forests today (Brewbaker, personal comm.).

Research on koa can date back to the 19th century. Early research had focused on botany, ecology, forest management, reforestation, insects and diseases, and vegetative propagation. With the erosion of koa genetic diversity and dramatics decrease of koa forests, genetic improvement of koa has been put on research agenda for many years, and is still being carried on.

General Biology

Distribution

Koa is distributed on all major Hawaiian Islands—Kauai, Oahu, Molokai, Maui, Lanai, and Hawaii, whose longitudes range between 154° W and 160° W, and whose latitudes range between 19° N and 22° N. It grows on volcanic soils of all geologic and degrees of development (Whitesell, 1990). Two main factors, the elevation and rainfall, limit the distribution of koa. Most koa grows in the high rainfall areas receiving 1,900 to 5,100 mm rainfall annually. Koa does not grow well in areas where rainfalls are out of that range. Most koa is concentrated at elevations between 600 to 1,800 m. Between 800 to 1,600 m is so-called "koa belt" where koa thrives (Whitesell, 1990; Sun, 1996).

Taxonomy and Evolution

Koa is a member of the thornless, phyllodinous group of *Acacia* subgenus *Heterophyllum* (Guinet and Vassal, 1978; Whitesell, 1990; Wagner et al., 1990). The taxonomy of koa has been ambiguous. Hillebrand (1884) classified native Hawaiian koa into three species: *A. koa*, *A. koaia*, and *A. kauaiensis* based on morphological variations in flower and pod. Rock (1920) further identified two varieties within *A. koa*: *A. koa* var.

lanaiensis and *A. koa* var. *hawaiiensis*. The former variety is distributed on Lanai, and the latter is only found on Hawaii with broad, straight phyllodes. St. John (1979) presented another classification of koa after reviewing specimens and the research of previous workers. In his classification, there were still three species as proposed by Hillebrand, but the varieties of *A. koa* were reclassified. Three varieties, *A. koa* var. *koa*, *A. koa* var. *waianaeensii* and *A. koa* var. *latifolia*, were proposed based on morphological variations in phyllodes and pods. *A. koa* var. *koa* can be found on all major Hawaiian Islands, and *A. koa* var. *waianaeensii* grows both on Hawaii and Oahu (most commonly on the Waianae Range); while *A. koa* var. *latifolia* (syn. *A. koa* var. *latifolia*) is located in the rain forests of Hawaii at high elevations. More recently, Wagner et al. (1990) disputed the previous delimitation of the species and favored a single species *A. koa* because of new morphological and flower developmental evidence. This classification has been widely accepted by koa research society (Hobdy et al., 1991). Genetically controlled variations in plant morphology suggest that it is still appropriate to reconsider the status of *A. kauaiensis*, and some varieties of *A. koa* recognized by St. John (Sun, 1996; Daehler et al., 1999).

The origin of koa is not clearly known. Koa is very morphologically similar to *A. heterophylla*, a species from Mascarene Islands in the Indian Ocean. Both species might have evolved from the same ancestor, possibly *A. melanoxylon* (Rock, 1920; Carlquist, 1965; Wagner et al., 1990). Recent work suggested that *A. heterophylla* is an autotetraploid of *A. melanoxylon* (Coulaud et al., 1995). Clear relationships between *A. koa* and *A. melanoxylon*, *A. koa* and *A. heterophylla* are still unknown. Isozyme analysis suggested that a third species, *A. simplicifolia* from Fiji, might be related to koa, with the

evidence of close band pattern with *A. koa* among 13 *Acacia* species (reviewed by Sun, 1996).

Cytology

Limited cytological studies revealed that *A. koa* is a tetraploid with a chromosome number of 2n=52 (Atchison, 1948; Carr, 1978). No variations in chromosome structure and number within the species are known. There are two ploidy levels, 2n=26 and 2n=52, in subgenus *Heterophyllum*. It seems reasonable that a tetraploid species like *A. koa* might have evolved from a diploid species through polyploidization. A recent study indicated that *A. heterophylla*, a relative of *A. koa*, might be an autotetraploid of *A. melanoxylon* (Coulaud et al., 1995). Regarding the high similarities between *A. koa* and *A. heterophylla*, a close cytological study involving the three species might provide some evidence of the evolution of *A. koa*.

Breeding system

Breeding programs can be misled without accurate information on reproductive biology. The estimation of genetic parameters such as heritability depends on knowledge of the breeding system (Bernardo, 2002). A successful breeding strategy should also be guided by accurate knowledge of the breeding system (self-crossing, outcrossing, or both) (Zobel and Talbert, 1984). Breeders always carefully employ different methods dealing with species with different breeding systems, especially approaching to advanced generations when the parents of seed orchard are to be selected (Zobel and Talbert, 1984).

Koa is believed to be entirely outcrossing (Sun, personal comm.), but results tend to contradict each other. The koa flower is dichogamous, with anthers maturing about 5 days prior to the stigmas (Brewbaker, 1977). This system prevents self-pollination in individual flower heads, though it cannot prevent cross-pollination among flowers within the same tree (Sun, 1996). Sun's (Sun, personal comm.) limited hand pollinations strongly indicated that self-pollination was impossible in koa, at least in trees he pollinated. Early work of Lanner (1965) indicated that koa is cross-pollinated by insects. The pod-set rate was low when the insects were excluded from the flowers, in contrast to a high fruiting rate when the flowers were open to the insects. Brewbaker (1977) assessed isozyme patterns and suggested that koa is partially self-pollinated. As a tetraploid species koa is likely to be self-compatible, as evidenced by its relatives in the genus *Leucaena*, where polyploids are usually self-fertile, and diploid are self-sterile (Brewbaker, personal comm.). A thorough examination on koa breeding system is still needed to clarify all controversy and to provide solid evidence for future breeding work.

Genetic variation

Phenotypic variations among koa populations are manifest in inflorescence and flower structure, phyllode and pod shapes, seed characters, seed arrangement, and growth habits. These were recognized by botanists such as Rock, Hillebrand, and St. John, and led them to classify koa into three species and several varieties. More recent studies revealed that a lot of morphological variations of koa are often caused by genetic factors. Sun's (1996) work showed that the growth rates (height and DHB) and phyllode development of koa were significantly different among the accessions, which were

represented by seeds from single mother trees, in seven progeny tests. Daehler et al. (1999) conducted a study on genetic variations of koa planted in two sites of Oahu in morphology and growth character. Their results strongly suggested that phenotypic differences in phyllode shape, extrafloral nectary morphology, and other characters such as plant height, branch coloration, forking, and juvenile leaves among accessions have a genetic base, and such genetic diversity might have ecological significance. From the view of koa genetic improvement and breeding, genetic diversity of the species is the basis on which superior lines can be selected (Daehler et al., 1999; Sun, 1996). Isozymic survey of koa populations also revealed high allelic variations in isozyme loci (Brewbaker, 1977; Conkle, 1996). Six isozymic genes studied by Conkle all showed polymorphic band patterns, and average alleles per gene ranged from 3 to 7. The average heterozygosity of isozyme genes was 0.41. Calculated genetic distance suggested that populations from Oahu, Maui, and Kauai are closely related, while populations from Hawaii are substantially different from other populations in terms of allelic type and frequency (Conkle, 1996).

Genetic improvement

Koa planting can date back to the 1910s (Skolmen et al., 1991), but little work on koa genetic improvement has been done. Koa plantations on public and private lands such as Bishop Estate were established from bulked, non-selected seeds from various sources (Skolmen et al., 1991). Very little genetic improvement of koa took place before the 1990s. An exception is the USDA Forest Service study that identified 52 "superior" trees with straight boles (Skolmen, 1977). A small progeny trial was planted at

Keanaolu, Hawaii, with seeds and clones derived from those trees (Skolmen et al., 1991). No quantified genetic variations were estimated, and no further selection took place (Sun, 1996).

A serious breeding program of koa was started in 1991 including seeds collected as early as 1960s by Brewbaker (Brewbaker, personal comm.). Since 1991, progeny trials have been planted annually at Hamakua, Hawaii Island. Each trial usually consisted of about 50 families in two replications, and was planted at 1 x 1.5 m spacing. Several trials were duplicated at Maunawili, Oahu with the same experimental design. Two seed orchards have been set up at Hamakua based on seeds of selected families with good performance (Sun, 1996; Brewbaker, personal comm.). In addition, researchers in Hawaii Agricultural Research Center (HARC) also conduct similar progeny trials at their research stations, including Maunawili (Dudley, personal comm.). All those trials enable researchers to quantify genetic variations, to select good families for commercial plantations, and to advance further breeding cycles. Much information on genetic diversity has been obtained through those trials.

Koa wilt

Many pathogens and insects impact on koa forests. Jones et al. (1991) summarized that 101 species of insects and 128 pathogens that can infect koa trees. Most of those organisms are not lethal. However, recently, a so-called "koa wilt" disease has raised deep concerns of foresters and breeders. This poorly understood disease causes severe mortality on nature koa forests as well as on artificial plantations, impacts trees of all size classes, and occurs on all major islands (Anderson, et al., 2002). Koa wilt was

first observed nearly 25 years ago (Gardner, 1980). The symptoms are preceded by phyllode yellowing and leaf defoliation, and subsequently the entire crown of an affected tree becomes wilted and dead (Anderson, et al. 2002).

Gardner (1980) isolated a seedborne fungus, *Fusarium oxysporum* f. sp. *koae*, from seeds of wilted trees. The pathogenicity of *F. oxysporum* was then verified on seedlings in the greenhouse. Inoculation of this fungus caused the wilt of young koa seedlings. It is believed that this fungus is also responsible for rapid death ("sudden decline") of old koa trees (Gardner, 1980). However, inoculating the pathogen back to field trees failed to develop wilt symptoms after eight months (Anderson et al., 2002). Factors such as the availability of soil moisture and pH may also play a predisposing role in koa wilt (Anderson and Gardner, 1998; Anderson et al., 2002).

Fusarium oxysporum typically spreads in the soil and invades the roots of koa trees, goes through and plugs the vascular system of plants and causes rapid death with wilt symptoms (Anderson and Gardner, 1998). Anderson et al. (2002) characterized the ecological and pathological characters of koa stands affected by wilt disease. The research found that the soil from dieback (wilt) stands had higher water contents and was more acidic than that from neighboring healthy stands. The disease apparently affects the physiological activities of affected trees, causing reduced area, mass, and mass to area ratio of phyllodes on epicormic shoots of the affected trees (Anderson et al., 2002).

Koa wilt is epidemic in progeny trials at Hamakua, Hawaii Island. Each year around 10% of trees in trials die, mostly from this disease. Genetic variations in resistance to the disease have been evident (Brewbaker, personal comm.). Certain families have consistent resistance or tolerance to the disease, while others are more

vulnerable. In the future, research on koa wilt should be a priority due to its severe causes to koa forests and plantations.

Vegetative propagation

Vegetative propagation plays an important role in forest tree breeding (Zobel and Talbert, 1984). A successful vegetative propagation method will enable breeders to capture both the general combining ability and specific combining ability during breeding cycles. One of such applications is the clonal seed orchard using cloned superior trees. Genetically identical clones are also useful in G x E tests.

The means of vegetative propagation of koa have been experimented with limited success. Skolmen (1977) conducted an extensive study on koa vegetative propagation. Both stem cutting propagation and air-layering of juvenile shoots produced roots successfully. No success was achieved in stem cuttings at phyllodinous stage. Both grafting and root cuttings were unsuccessful. Rooting ability varied from tree to tree, indicating the involvement of genetic factors. Root suckers were the only suitable material for vegetative propagation at mature stage. Rooted plantlets survived poorly. Tissue culture of koa was also tried by Skolmen (1977). Callus induction was successful, as well as the regeneration of shoots from the callus. Meristematic multiplication using young koa seedlings seemed promising in another study conducted by Nagai and Ibrahim (1996). Despite the success achieved in those studies, techniques for koa cloning are still not ready to produce a large amount of koa clones and to clone those "elite" trees from the wild. More research is required to refine and develop cloning techniques.

CHAPTER 6. CYTOLOGY OF KOA POPULATIONS

Introduction

Acacia koa Gray is an indigenous legume species growing on all major Hawaiian Islands. The morphology of koa is highly variable from population to population. It was once classified into three species and several varieties (Hillebrand, 1884; Rock, 1920; St. John, 1979). The previously recognized koa species were *A. koa, A. koaia*, and *A. kauaiensis*. Such recognition was disputed by Wagner et al. (1990), who emphasized the existence of intermediate forms of the proposed species. They also noted that some characters of *A. kauaiensis*, such as separate petals, were not unique. The authors treated those variants as three subspecies, rather than as three species, a position widely accepted by koa researchers (Hobdy et al., 1991). For convenience in this study, the three types of koa would be designed by their published species names *A. koa*, *A. koaia,* and *A. kauaiensis* without endorsement of any taxonomy. Collectively the three kinds of koa will be referred to simply as "koa".

Koa populations dominate the forests of all islands, and vary in many aspects like growth habit and plant morphology. *Acacia koaia* grows in dry areas of Molokai, Lanai, Maui, and Hawaii. It has a shrubby habit, significantly harder wood, narrow pods, and a longitudinal seed arrangement. *Acacia kauaiensis* has large round seeds, separate sepals and petals, and a terminal inflorescence that are said to distinguish it from *A. koa* and *A. koaia*. It is found only on Kauai. *Acacia kauaiensis* and *A. koa* are more arboreal than *A. koaia*. Major distinctions of three types are summarized in Table 6.1.

Figure 6.1 shows seed and leaf variations of koa. Very large phyllodes resemble those of the *A. kauaiensis*, and very thin phyllodes characterize *A. koaia*.

Table 6.1. Major distinctions of three koa types.

Type	Tree form	Inflorescence	Pod	Seed
A. koa	Arboreal	Axillary	Vertical seed arrangement	Variable size, oblong and long
A. koaia	Shrubby	Axillary	Longitudinal seed arrangement	Small, long
A. kauaiensis	Arboreal	Terminal	Vertical seed arrangement	Big, round

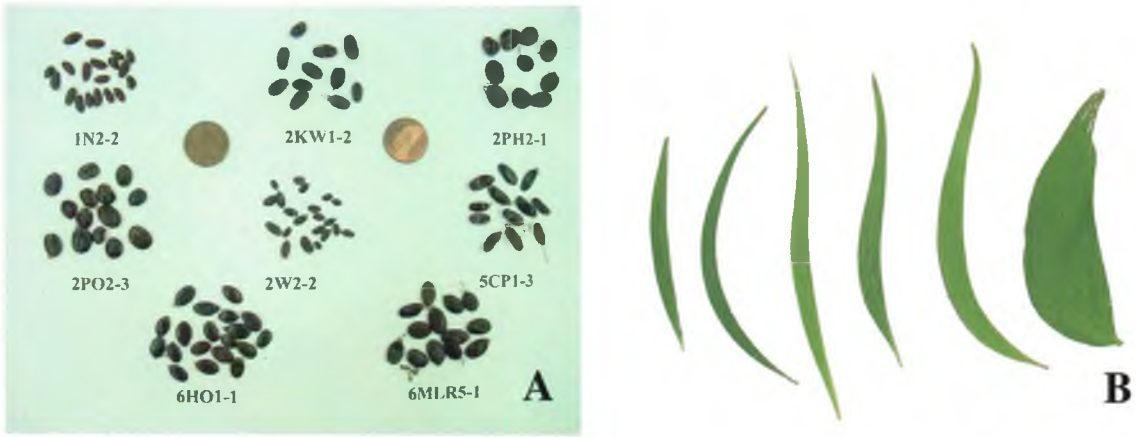

Figure 6.1. Variations of seed (A) and leaf (B) of *Acacia koa*.

The chromosome number of koa was reported to be 2n=52 by Atchison (1948) and later confirmed by Carr (1978). Both workers reported the chromosome number of koa as part of a more general survey; their studies were not extended to the highly variable populations within the species, and no specific studies of chromosome morphology or karyotype were involved in their research.

Cytological variation (polyploidy) among three types of koa has been suspected (Brewbaker, personal comm.) based on observed variation in seed size of three taxa.

The objectives of this study included:

1) refining techniques to optimize cytological study of koa;
2) detection of possible variations in chromosome number among koa populations;
3) morphological characterization of chromosomes of koa.

Materials and methods

Materials

Koa seeds from previous collections were selected for this study. Those seeds included three botanical types that have significant morphological variations, and are suspected to be in different ploidy levels. For each type, seeds from different islands representing different populations were examined.

Seeds were nicked at cotyledon side to facilitate germination, and were germinated in petri dishes with wet tissue paper at room temperature (~23 $^{\circ}$C). After seed germination, detached or intact roots (0.1 to 1.5 cm in length) were examined for

mitotic configurations. Carnoy's fixative (3 parts of 95% ethanol: 1 part of acetic acid (v/v)) was used to fix all materials after being pretreated as described below.

Pretreatment

Detached root tips or intact germinating seeds of *A. koa* were treated with five pretreatment solutions. Pretreatment solutions cause chromosome contraction, thus facilitating chromosome spread, and arrest cells in metaphase, thus increasing the number of mitotic cells. The solutions included 0.1% colchicine, 0.001 M 8-quinolinol, 0.001M 8-quinolinol + 0.1% colchicine (Ma, et al., 1996), 300 ppm cycloheximide (modified from Wang et al., 1999), and saturated para-dichlorobenzene solution (PDB) (Motley and Carr, 1998), all in aqueous form. Different treatment durations from 0.5 to 5 hours were assessed.

Digestion and slide preparation

Two digestion and preparation methods were used in the study—an acid digestion and an enzymatic digestion. The acid digestion method used 1: 1 HCl : 95% ethanol (v/v) mixture to soften the materials. First, a small portion of fixed root tip (~0.5 mm) was washed in pure water and then placed on a clean slide. The tissue was digested by acid-ethanol mixture for 5 minutes. After digestion, the material was washed with water. Then a drop of stain was added and was left for about 30 seconds. Excess stain was then blotted away, and the tissue was carefully macerated and smeared with a scalpel. A cover slide was placed on the squashed tissue. Finally, the slide was turned over (cover slip down), placed on tissue paper, and pressed firmly with the thumb.

The enzymatic digestion was carried out in 5 root tips at a time. These were washed and transferred to a 1-ml tube to which 0.5 ml of mixed enzyme solution (5% cellulase and 2% pectinase, Sigma) was added. Root tips were digested for 5 hours at room temperature (~23 ^{o}C). After digestion, root tips were washed again with water prior to 200 µl Carnoy's fixative solution. The tissue was gently dissociated with a pair of forceps until no large pieces remained. The tissue suspension was then centrifuged at 5000 rpm for 5 minutes. After the supernatant was discarded, the pellet was washed once with water prior to the addition of another 200 µl Carnoy's fixative solution. The pellet was then resuspended by shaking the tube. For slide preparation, 3-5 µl of the tissue suspension was transferred to a clean microscope slide and left to air-dry overnight in a hood. A drop of stain was then added to the dried sample on the microscope slide and a cover slip applied prior to observation under the microscope.

Stains

Carbol-fuchsin, aceto-carmine (1% carmine in 45% acetic acid), and silver nitrate stain were tried. To observe the nucleolus and NOR (nucleolus organizer region), root tips were first squashed in 45% acetic acid. The cover slip was then removed in liquid nitrogen, and the slide was air-dried. After drying, a drop of freshly prepared silver nitrate solution (1g silver nitrate dissolved in 1ml sodium citrate buffer) was added to the slide. The slide was then incubated in a moist chamber at 60 ^{o}C for 30 minutes. After incubation, the slide was rinsed in water and observed under the microscope.

Microscope observation and photographing

All slides were observed under a Carl Zeiss phase contrast microscope. Cells with good chromosome spreads were photographed with a Nikon digital camera mounted directly on the microscope. Photographs were taken using the 40 x or 100 x objective lens and a 10 x eyepiece in front of the camera lens.

Results

Techniques of chromosome preparation

Length of roots

Mitosis was observed in roots at all lengths. However, if the roots were short (<0.5 cm), a number of unknown stainable particles were present in root tip cells that could be confused with chromosomes. These particles might be storage starch or protein. The particles disappeared after the root grew to a certain length, possibly because they were consumed by the cells during root extension. Therefore, choosing longer roots (~1.5 cm) significantly improved slide qualities.

Pretreatment

Ratings of effectiveness of six solutions on chromosome contraction and spread are summarized Table 6.2. Only the treatment with 300 ppm cycloheximide produced satisfactory effects on chromosome contraction and spread through all four treatment durations (2 to 5 hours). The treatment with 0.001 M 8-quinolinol had a good effect on chromosome spread and contraction, but 300 ppm cycloheximide was superior. Other chemicals either had little effect on chromosome contraction or caused chromosome

stickiness (Figure 6.2). Sticky chromosomes were especially found in root tips treated with 0.1% colchicine. A similar result was reported by Bukhari (1997b) on other *Acacia* species. Even with the pretreatment using 300 ppm cycloheximide some evidence of stickiness might be noted (Figure 6.3). Water and PDB had no apparent effects on chromosome contraction and spread. Therefore, cycloheximide was used exclusively for the remainder of this study.

Table 6.2. Effects of five pretreatment agents on chromosomal contraction and spread after different treatment durations.

Duration (hours)	Water		Colchicine		Cycloheximide		8-quinolinol		8-quinolinol +colchicine		PDB	
	Con.	Sprd.	Con.	Sprd.	Con.	Sprd.	Con.	Sprd.	Con.	Sprd.	Con.	Sprd.
2	+	+	++	+	+++	++	++	++	++	++	+	+
3	+	+	+	+	+++	+++	+++	+	++	+	+	–
4	+	+	+	+	+++	+++	+++	+	++	+	+	–
5	+	+	+	+	++	+++	+++	+	++	+	+	–

Con.: Chromosome contraction.
Sprd.: Chromosome spread.
+: Fair.
++: Good.
+++: Excellent.

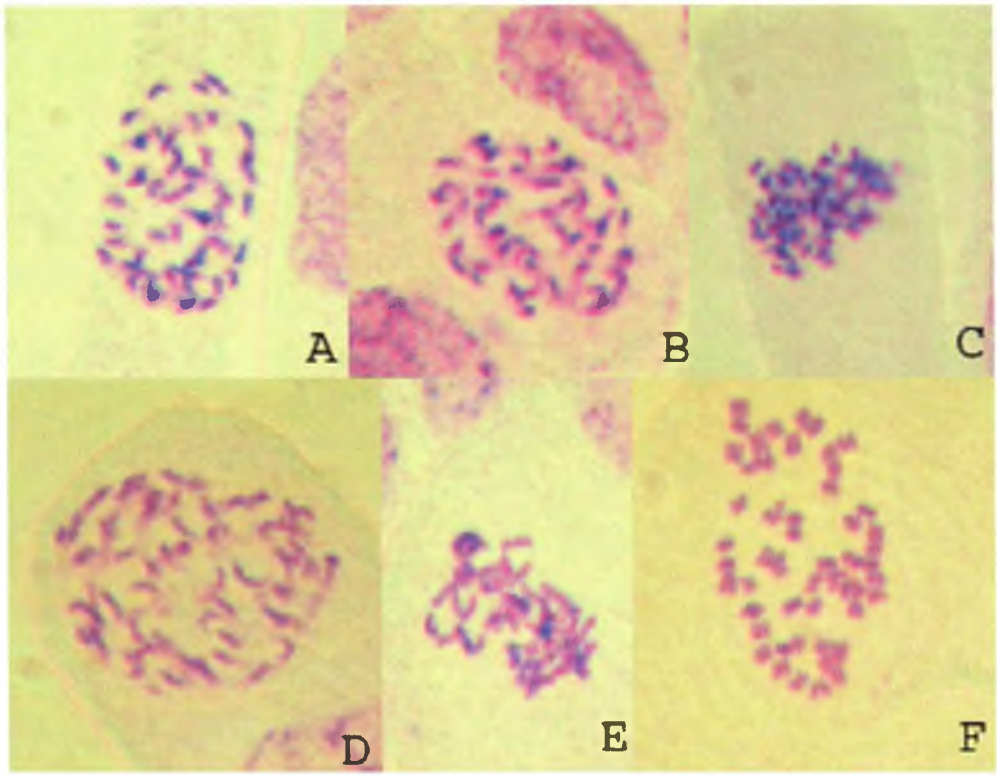

Figure 6.2. Root tip cells of *Acacia koa* treated with 2 hours of A: 0.001M 8-quinolinol; B: 0.001M 8-quinolinol +0.1% colchicine; C: 0.1% colchicine; D: saturated PDB; E: water; F: 300 ppm cycloheximide. All figures x 400.

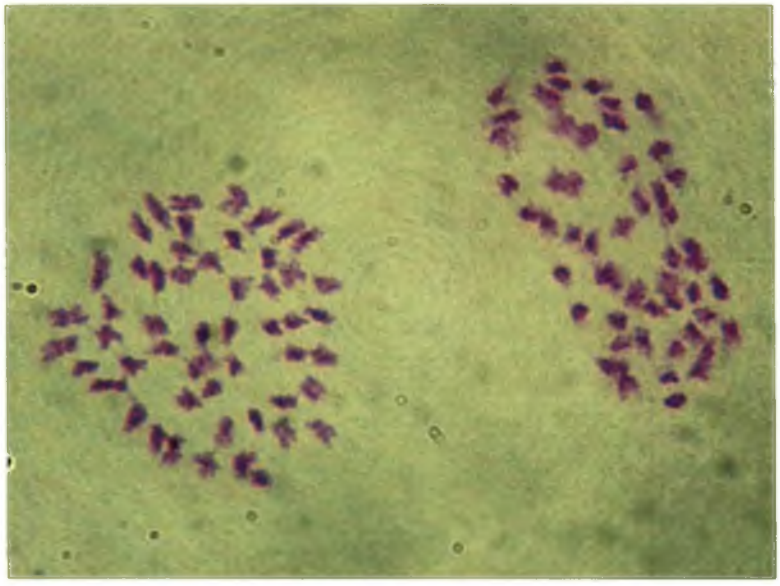

Figure 6.3. Metaphase cells of *Acacia koa* root tips, showing stickness of chromosomes. x 1000.

Pretreatments of 2 to 3 hours in 300 ppm cycloheximide at room temperature led to significant chromosome contraction and good spread for determination of chromosome numbers. However, chromosome structure was not clear. Chromosomes became fuzzy if the pretreatment duration was prolonged to 5 hours or more. Short treatment durations (40 to 90 minutes) led to good chromosome configurations that revealed some detailed structure of chromosomes.

Digestion and slide preparation

Both digestion methods yielded good cell separation and spread. However, the enzymatic method was more time-consuming (>5 hours) and complex, and sometimes the chromosomes were damaged due to long durations of enzymatic treatment. Thus, only HCl-ethanol method was used in most observations.

Stains

Carbol-fuchsin produced better staining results than acetocarmine. Chromosomes were stained a deep purple color by carbol-fuchsin, with little stain in the cytoplasm. Thus a sharp contrast of chromosomes and cytoplasm greatly improved the quality of photographs (Figure 6.4). Aceto-carmine-stained chromosomes were only a light red color. The color was so light that it was difficult to photograph and to observe chromosome structure (Figure 6.4).

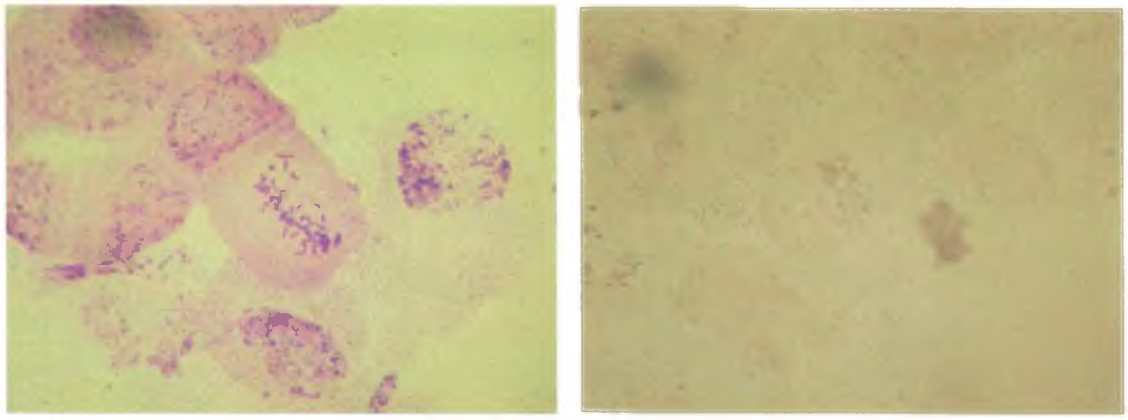

Figure 6.4. Root tip cells of *Acacia koa* were stained by carbol-fuchsin (left) and by acetocarmine (right)--chromosomes were better stained by carbol-fuchsin. x 400.

Cytology of *A. koa* somatic cells

Chromosome numbers of koa

Chromosome numbers of populations from three types of koa are presented in Table 6.3, and Figure 6.5. All studied populations (belonging to all three types of koa) had 2n=52 chromosomes. The results confirmed the observations of Atchison (1948) and Carr (1978). No variations in chromosome number were found.

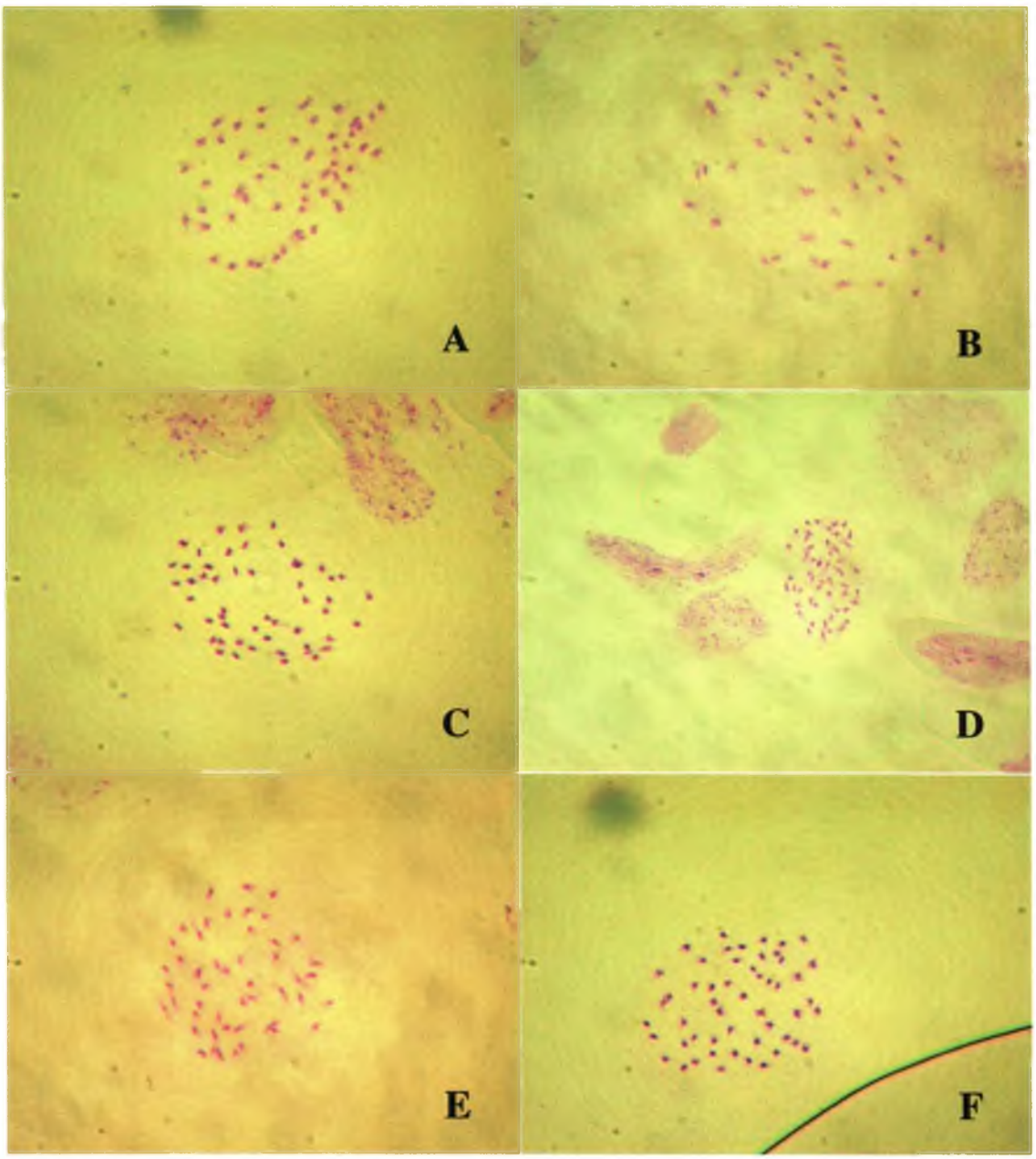

Figure 6.5. Chromosomes of six families of koa. All families had 2n=52 chromosomes. A: 2HELEALA1, *A. koaia*. B: F45P5, *A. koa*. C: 95-130-1, *A. koa*. D: 6HO3-1, *A. koa*. E: 2MA3-1, *A. kauaiensis*. F: 1OPR1, *A. koa*. All figures x 400.

Table 6.3. Family, seed morphology, and chromosome number of koa.

Type	Family	Seed morphology	Chromosome number
A. koaia	6KOIA1-4	Small, round	52
	2HELELEA1	Small, oblong	52
A. kauaiensis	2MA2-1	Big, round	52
	2MA3-1	Big, round	52
A. koa	1OPR-1	Long, medium	52
	2PK-5	Long, medium	52
	2PK2-1	Long, medium	52
	2MI3-2	Long, big	52
	6KE1-1	Oblong, big	52
	6HO1-2	Oblong, big	52
	6UMLC	Oblong, big	52
	95-130-1	Long, medium	52

Morphology of the chromosomes

A definitive study of chromosome morphology at metaphase was deemed impractical in *A. koa* due to the smallness of the chromosomes and unclearness of the centromeres. Some morphological differentiations among chromosomes at prometaphase could be observed. However, they were very difficult to quantify because chromosomes were not always straight, and often overlapped.

Chromosome morphology was also extremely difficult to resolve due to small chromosome size. Variation in chromosome size could be observed in early to late prometaphase, but all chromosomes looked similar in size at metaphase. Several long chromosomes could be identified at early to late prometaphase, while most of the chromosomes were relatively short. It was very difficult to match homologous chromosomes because of high variability of chromosome morphology from slide to slide and the lack of clear distinction of centromeres. For these reasons detection and

characterization of any cytological variation that may exist among populations and types considered here are beyond the scope of this research.

At least one pair of chromosomes with secondary constrictions could be identified in some cells, while only one chromosome with a secondary constriction was visible in other cells (Figure 6.6. C, D). Generally, chromosomes with secondary constrictions could be observed in very few cells.

Nucleolus

One big nucleolus was visible in interphase and prophase of mitosis in most cells. Occasionally, one big and one small or two small nucleoli were observed (Figure 6.6. A, B).

Discussion

Chromosome preparation of *Acacia* species has been reported to be difficult. Several authors have experimented with pretreatment chemicals and methods to achieve satisfactory contraction and spread of the chromosomes. Bukhari (1997b) found that treatments of colchicine and 8- quinolinol with 15 to 240 minutes resulted in condensed and sticky chromosomes in six *Acacia* species, whereas treatment in water at 20 °C led to good chromosome contraction and spread. The author also found that chromosomes from ruptured cells were larger than those from intact cells. Coulaud et al. (1995) found that 0.002 M 8-quinolinol at 16 °C could improve the spread of chromosomes of *A. heterophylla* and *A. melanoxylon*. Other pretreatment chemicals such as colchicine, 1-

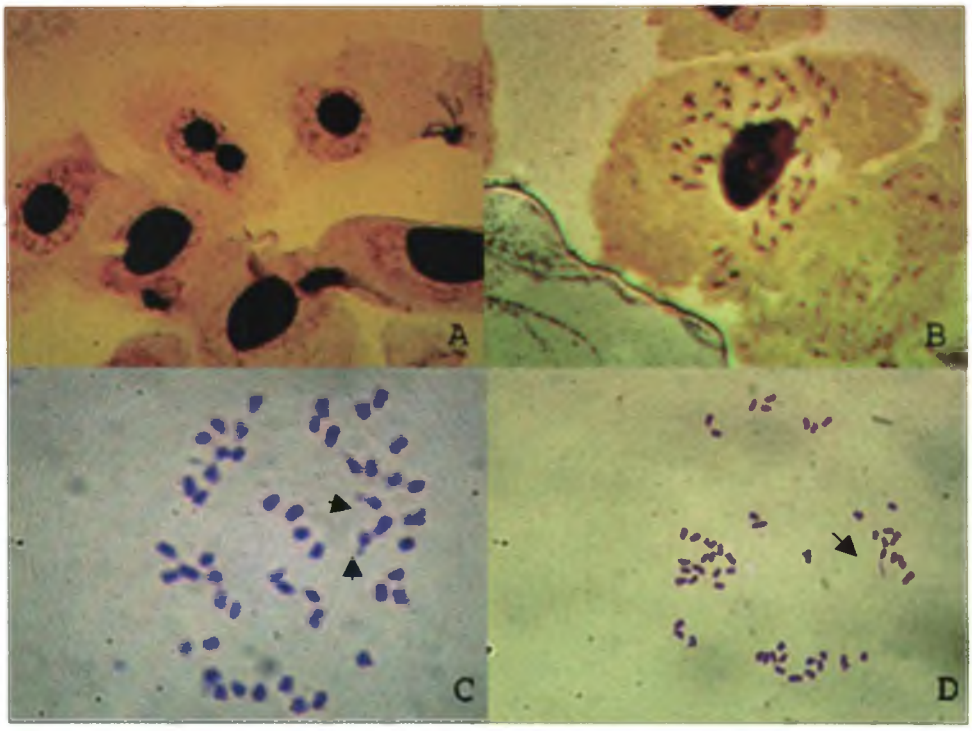

Figure 6.6. Mitosis in root tip cells of *Acacia koa* showing nucleoli and chromosomes with secondary constrictions. A: Interphase cells showing either one nucleolus or two nucleoli. B: A prophase cell with one large nucleolus. C: Putative pair of chromosomes (arrows) each with a secondary constriction. D: One chromosome (arrow) with a secondary constriction. All figures x 1000.

chloronaphthalene, oryzalin, cold water, or combinations thereof produced no good chromosomal spreads. In the present study colchicine caused very sticky and clustered metaphase chromosomes in *A. koa*, a result comparable to those of Bukhari (1997b) and Coulaud et al. (1995). Treatments of 0.002 M 8-quinolinol worked well in Coulaud et al.'s study, but not in Bukhari's study; it also worked well for *A. koa*, but 8-quinolinol was not the best treatment in my study. In contrast to what was achieved by Bukhari, the pretreatment with water at room temperature (~23 ^{o}C) did not result in chromosome contraction and did not enhance chromosome spread at various durations in my study.

Cycloheximide was the best pretreatment agent for koa. After a short treatment of 300 ppm cycloheximide (3 to 4 hours), the chromosomes of koa were highly condensed into short rods at metaphase. Such rod-like chromosomes were well spread after proper pressure on cover slips and were very suitable for chromosome counts. Shorter treatments (1 to 2.5 hours) produced less condensation of metaphase and prometaphase chromosomes. Structural details such as centromeres and ratios arm lengths of some of the large chromosomes could be observed.

Cycloheximide is a protein synthesis inhibitor that blocks the synthesis of spindle formed at metaphase, and thus arrests cells at metaphase (Wang et al., 1999). This pretreatment also results in increased chromosome contraction that facilitates spread of chromosomes on cytological preparations. Although cycloheximide is widely used in the research of plant and animal cells, its applications in cytological study are rare. In addition to its successful application in koa cytology, cycloheximide also worked well in *Leucaena* (see Chapter 4)

No variation in ploidy level was found in koa. Variation in ploidy level in the genus *Acacia*, however, is very common. About 70% of the species are diploid, while the rest are polyploids (Brewbaker, 1990). There are four ploidy levels in the genus. The most common number is 2n=26, while the numbers of 2n=52, 78, and 104 are also found in certain species. Different ploidy levels are found in the same species of *Acacia*, such as *A. tortilis*, which has two ploidy levels, tetraploid (2n=52) and octoploid (2n=104) (Bukhari, 1997a).

At least one pair of chromosomes with secondary constriction could be observed in *A. koa* (Figure 6.6 C, D) at late prometaphase. Coulaud et al. (1995) reported at least two pairs of chromosomes had secondary constrictions in *A. heterophylla* and one pair in *A. melanoxylon*, species that are tetraploid and diploid respectively. *Acacia heterophylla* is closely related to *A. koa*. There are at least two explanations for variations in the number of chromosomes with secondary constrictions in *A. koa*. One is that there are more than one pair of chromosomes with secondary constrictions, but due to limitation of technique, only one pair was observed. Another possibility is that there is indeed only one pair of chromosomes with secondary constrictions. Since *A. koa* is a tetraploid, the presence of only one pair of chromosomes with secondary constrictions might imply that the species is an allotetraploid, which evolved from two distinct species. As found in *Allium* (Langer and Koul, 1983), allotetraploid species sometimes have fewer chromosomes with secondary constrictions than would be expected for their ploidy level due to the phenomenon called amphyplasty. In such cases, the activity of NORs located on the secondary constrictions from one species was suppressed by the NORs from the other species. Thus fewer secondary constrictions can be observed due to the inactivation

of some of the NORs. Even different populations of the same species may show this effect as found in *Calycadenia* (Carr, 1975).

Work on NOR chromosomes using *in situ* hybridization in koa and its relatives may help interpret relationships among those species as accomplished by Bennett et al. (1992) in their study of the allopolyploid origin of *Milium montianum*. NORs are loci on chromosomes encoding rRNAs, and their distribution is species specific. Therefore, species relationships might be revealed if the information of NORs of all related species were known.

Conclusions

The chromosome number of all populations of koa determined was 2n=52. No variations in chromosome number were found among three koa types and populations. The observation of morphology and structure of koa somatic chromosomes was generally difficult. The application of pretreatment of cycloheximide significantly improved the contraction and spread of root tip chromosomes, and further facilitated chromosome counts and morphological observation with the help of effective carbol-fuchsin stain. The characterization of chromosome morphology was difficult due to the small size of chromosomes. A maximum of one putative pair of chromosomes with secondary constrictions and up to two nucleoli were found in root tip cells of *A. koa*.

CHAPTER 7. VEGETATIVE PROPAGATION OF KOA BY CUTTINGS

Introduction

Koa genetic improvement, initiated more than a decade ago, aims to provide better-adapted materials for koa reforestations. Vegetative propagation can be an important part of genetic improvement programs and includes such methods as tissue culture, cutting, grafting, and air-layering. If used wisely and properly, the progress of species improvement can be greatly accelerated. Vegetative propagation could be useful for propagating selected koa trees with superior growth and stem form, high resistance to diseases and insects, desirable wood characteristics such as curly or fiddle-back grains. Vegetative propagation provides genetically identical materials for commercial plantations as well as for testing genetic by environment interactions. Another possible use of vegetative propagation is for germplasm conservation and seed orchards by propagating elite trees.

Koa vegetative propagation was studied intensively by Skolmen (1977) who evaluated different methods of tissue culture, grafting, cuttings, and air-layering. Regeneration of plants from calli and embryoids induced from seeds germinated *in vitro* was successful. Organogensis of shoot tips was not successful. Grafting was extremely difficult without any success. Rooting succeeded under mist using stem cuttings with true (juvenile) leaves treated with various nutritional and auxin solutions. A similar result was obtained on air-layering, where only shoots with true leaves could root. Root cuttings failed to produce any roots. The research indicated that only materials at juvenile stage could produce adventitious roots in koa. The author realized that the

importance of juvenility of propagation materials, but attempts to induce juvenile shoots were not successful. More recently, *in vitro* propagation of *A. koa* was studied by Nagai and Ibrahim (1996). Shoot and stem culture using young seedlings indicated feasibility according to early results.

Research on cutting propagation of other *Acacia* species is extensive. Ahmad (1991) reported on rooting of stem cuttings in *A. mangium*, noting that the rooting ability of stem cuttings was a strong function of the age of stock plants. Mean rooting percentage of 6-month stem cuttings was 71%, whereas the percentage of 24-month cuttings decreased to 15%. The presence of a phyllodinous leaf on the cutting was an important factor for high rooting percentage. Auxins such as IBA and NAA in various concentrations increased rooting frequencies. Another study in *A. auriculiformis* found that cuttings from epicormic shoots (true-leaf shoots) rooted better than those from phyllodinous shoots (Simsiri, 1991). Cuttings treated with a commercial rooting compound "Seradix" No.3, which contained 0.8% IBA (indo-3-butyric acid) also gave better rooting results. The study also revealed that the age of tree greatly affected the root ability of cuttings, and only cuttings from trees younger than 10 years old could root. A study conducted in China (Chen and Li, 1997) reported that seasonal factors greatly affected rooting of stem cuttings from coppiced *A. mangium*, *A. auriculiformis*, and *A. crassicarpa*. Cuttings rooted well in a non-mist rooting bed only in fall, and rooting compounds also significantly improved the rooting of cuttings.

The objectives of this study were to characterize the rooting ability of koa stem cuttings at three developmental stages, to characterize the cause of rooting failure, and to

use plant growth regulators and other physiological treatments to improve the rooting of cuttings.

Materials and methods

General information

The development of koa trees can be divided into three stages: juvenile, transitional, and mature in terms of morphological and physiological changes. Seedlings only with true leaves were considered at juvenile stage. Trees that had fully developed phyllodinous leaves, but had not reached flowering stage were regarded at transitional stage. Trees that had started to flower were at mature stage. In this study, rooting experiments were classified into these three developmental stages to better understand the effect of cutting's maturity on rooting.

Koa cuttings at juvenile stage were taken from seedlings or subclones grown in the greenhouse of the Waimanalo Research Station. Cuttings at transitional stage were taken from trees growing in the field of the Waimanalo Research Station. Mature-tree cuttings were taken from three 8-year old trees at the Maunawili Research Station, and one 6-year old tree at the Hamakua Research Station. Rooting conditions of koa were similar to those of *Leucaena* hybrids (see Chapter 2). Rooting results usually were recorded after 6 to7 weeks of insertion to the medium.

Experiments on cuttings from juvenile seedlings or subclones

Experiment 1 was set up in June 2001. Four-month old juvenile seedlings were grown in the greenhouse. Rooting of cuttings from 7 families each with 5 trees was

tested in an unreplicated experiment. The number of cuttings of each tree varied from 4 to 20. All cuttings were single-node with entire true leaves.

Experiment 2 was set up in December 2001 to test the effect of age of stock plants on rooting. Subclones obtained in Experiment 1 were used in this unreplicated experiment. The age of plants was counted from the day when the subclones were transplanted from 2.5" pots to half-gallon pots. At that time, those subclones had three to four leaves. Subclones were used to eliminate variations caused by genetic effects and to maximize the effect of age. All cuttings were single-node, and had true or phyllodinous leaves.

Experiments on cuttings from trees at transitional stage

Experiment 3 and 4 were set up in March and April 2002 to test the effects of auxins on rooting of cuttings at transitional stage. All cuttings were collected from plants grown at the Waimanalo Research Station. The plants had fully developed phyllodinous leaves and an age of 14 months. All cuttings were trimmed to half phyllodinous leaves.

In Experiment 3 cuttings from trees of family 2W2-2 were quick-dipped in solutions of IBA (indole-3-butyric acid, acid form, dissolved in 50% ethanol), or NAA (α-naphthalene acetic acid, acid form, dissolved in 50% ethanol), and two controls (water, and 50% ethanol). Two types of cuttings were tested, bi-nodal stem cuttings and terminal shoot cuttings. Cuttings were inserted into the rooting medium after auxin solutions dried. The experiment was unreplicated, and there were 12 or 24 cuttings in each treatment.

In Experiment 4 cuttings from trees of family 2W2-2 were treated with IBA or NAA mixed in talc powder to prevent possible bleaching by water. Cuttings were first dipped into the 50% ethanol solution, and then into auxin-talc powder. Cuttings without any treatment or treated only with talc powder were used as controls. The experiment was unreplicated. Two types of cutting were tested, bi-nodal stem cuttings and terminal shoot cuttings. Each treatment contained 12 cuttings.

Another two experiments (Experiment 5 and 6) applied auxins or cytokinin to prevent leaf shedding of cuttings. Experiment 5 was conducted in May 2002, in which the leaves of single-node cuttings from a tree of family 6HO1-4 were sprayed with 10, 100, or 1000 ppm IAA (indole-3-acetic acid, acid form), NAA (acid form), IBA (salt and acid form), or 6-BA (6-benzyladenine) dissolved in 50% ethanol or water before insertion to the medium. Water and 50% ethanol were used as controls. All cuttings were prepared with half phyllodinous leaves. The experiment was unreplicated, and there were 12 cuttings in each treatment.

Experiment 6 was carried out in June 2002 to determine the effects of 6-BA and NAA on leaf retention and rooting in more koa families. In this experiment, leaves of bi-nodal cuttings from trees of 4 families at the transitional stage were sprayed with the combinations of 6-BA and NAA solutions before cutting sticking. The two solutions were sprayed separately. All cuttings were trimmed to half phyllodinous leaves and were dipped into 1000 ppm IBA solution first. The experiment was replicated three times, and there were 12 cuttings in each replication. After first spraying, all cuttings were sprayed again in second week. The numbers of cuttings that shed leaves were recorded every

week (totally 5 weeks). The number of rooted cuttings and the number of cuttings with leaf retention were recorded at the end of the experiment.

Etiolation treatments (Experiment 7) were applied to trees at transitional stage at the Waimanalo Research Station in January 2003. Terminal shoots of trees of 3 families--2W2-2, 5F45P5, and KOKEEL2--were first trimmed to induce new growth before etiolation treatments. When new sprouts started emerging after three weeks, the trees were covered with two types of structure. A small cage made from bamboo sticks and a black trash bag was used to cover pruned side branches. A large cage made from a frame of PVC pipes and black mulch cloth as covering material was used to cover an entire pruned tree in the field. The light interception rates of both types of cage were close to 100%. The temperature differences inside and outside of cages were about 6 ^{o}C on sunny days. All plants were treated in dark for 3 weeks.

New shoots were allowed to harden in full sun for 1 week after etiolation. Meanwhile, Velcro belts were applied to half of etiolated shoots during hardening. Single-node cuttings were collected from dark-treated shoots. Due to an insufficient number of cuttings, all experiments were unreplicated. No auxin treatments were applied to the cuttings.

Experiments on cuttings from mature trees

Two experiments (Experiment 8 and 9) were conducted to test the rooting of cuttings from mature trees. Experiment 8 was conducted in April 2002, in which bi-nodal cuttings from three 8-year old mature trees (2PK2-1) at Maunawili were treated

with quick dip of IBA or NAA solutions. All cuttings were prepared with entire phyllodinous leaves.

Experiment 9 was conducted on cuttings of a mature tree (6KMC1-5) from the Hamakua Research Station in May 2002. The tree was 6 years old and had started to set fruits. Single-node cuttings were treated with IBA (acid form, quick dips) or the combinations of IBA (acid form, quick dips) and 6-BA (sprays on leaves of cuttings) solutions. Water and 50% ethanol were used as controls. All cuttings were prepared with entire phyllodinous leaves. The experiment was unreplicated, and there were 12 cuttings in each treatment.

Results

Rooting of cuttings from juvenile seedlings or subclones

The results of Experiment 1 are summarized in Table 7.1. Cuttings from juvenile seedlings (four months old) rooted fairly well. The overall rooting frequency of seven families ranged from 31.2 to 68.3% with an overall rooting frequency of 52.2%. At least three families--5F45P5, 91-206-3, and 95-242-8--rooted better than other 4 families (average 68.2% of rooting vs. 40.2%). Variation within families was fairly large, but sample numbers were small. The variation was probably caused by the genotypes of individual trees as well as physiological state of cuttings.

Root qualities of rooted cuttings primarily depended on rooting period and post-rooting management (Figure 7.1). Cuttings with longer rooting periods and a period of hardening (reduced time of mist) usually had high rooting qualities and high survival rates after transplanting to potting mixture.

Subsequent rooting experiments using cuttings from the same seedlings were low in rooting frequencies. Three families (5F45P5, DTF1, and 6KOLO8) tested in August (6 months old) had an average rooting frequency of 20.8, 31.3, and 3.1% respectively, which were much lower than those of previous rooting (47.1, 73.1, and 39.0% respectively).

It was evident that the age of plants has caused low rooting ability. Experiment 2 was set up to test the effect of age on the rooting of cuttings during winter months using subclones obtained from the Experiment 1. Rooting results of cuttings from plants at age 3, 6, and 9 months are summarized in Table 7.2. The results indicated that the ages of stock plants greatly affected rooting ability of single-node cuttings harvested from them. All plants at the age of 3 months rooted 100%. Rooting frequencies declined substantially at the age of 6 months, and rooting ability was lost at the age of 9 months.

Table 7.1. Rooting after 6 weeks of one-node cuttings from individual seedlings of 7 families at the age of 4 months.

Family	Tree 1		Tree 2		Tree 3		Tree 4		Tree 5		Overall rooting %
	# of rooted	Total	# of rooted	Total	# of rooted	Total	# of rooted	Total	# of rooted	Total	
2DTF1	1	4 (25.0%)‡	10	20 (50.0%)	5	12 (41.7%)	11	20 (55.0%)	5	12 (41.7%)	47.1
5F45P5	10	12 (83.3%)	16	20 (80.0%)	6	8 (75.0%)	3	4 (75.0%)	3	8 (37.5%)	73.1
91-206-3	1	4 (25.0%)	3	8 (37.5%)	11	12 (91.7%)	8	8 (100.0%)	5	9 (55.6%)	68.3
95-242-8	6	11 (54.5%)	6	8 (75.0%)	6	6 (100.0%)	7	12 (58.3%)	8	12 (66.7%)	63.3
6KOLO8	3	4 (75.0%)	6	12 (50.0%)	1	8 (12.5%)	3	12 (25.0%)	4	8 (50.0%)	39.0
00-215-5	5	8 (62.5%)	0	4 (0.0%)	4	8 (50.0%)	0	8 (0.0%)	1	4 (25.0%)	31.3
91-218-9	8	12 (66.7%)	5	12 (41.7%)	10	12 (83.3%)	2	8 (25.0%)	0	4 (0.0%)	43.3
Average (%)											52.2

‡: Figures in parentheses are rooting frequencies of individual trees.

Table 7.2. Effects of ages of stock plants on the rooting of cuttings from 4 koa subclones.‡

Family	Rooting at the age of								
	3 months			6 months			9 months		
	# of rooted	Total	Rooting %	# of rooted	Total	Rooting %	# of rooted	Total	Rooting %
5F45P5-2	6	6	100.0	18	30	60.0	0	27	0.0
93-218-9-3	4	4	100.0	7	13	53.8	0	19	0.0
91-203-1-2	9	9	100.0	13	18	72.2	0	31	0.0
2DTF1-4	N/A	N/A	N/A	19	24	79.2	0	28	0.0
Average (%)			100.0			66.3			0.0

‡: All cuttings were from shoots produced after 40 days of pruning.

Figure 7.1. Koa juvenile cuttings rooted without auxin treatments.

Rooting cuttings from trees at transitional stage

Auxin treatments

Experiment 3 involved 6 concentrations (0.25, 0.50, 0.75, 1.00, 1.25, and 1.50%, w/v, quick dips) of IBA or NAA, which were applied to cuttings from plants at transitional stage (Table 7.3). The leaves of cuttings started to shed one week after cuttings' insertion into the medium. Only two stem cuttings (8.3%) rooted with the treatment of 0.25% IBA. No stem cuttings from other treatments as well as from the controls rooted. More terminal cuttings produced roots, especially from those treatments of NAA (0.5 to 1.25%). However, the increases in rooting frequency were not substantial in both two types of cuttings, and the effects of auxins were negligible. It was observed that all rooted cuttings had leaf retention during cutting propagation and produced some calli before root initiation, and all unrooted cuttings had shed leaves before they died.

Cuttings treated with auxin powder instead of solutions (Experiment 4) gave similar disappointing results (Table 7.4). Six different concentrations were used in the experiment. Most concentrations of auxin powder failed to improve the rooting of cuttings. Slight increases in rooting frequency were found in some treatments (2.25 and 3.25% of IBA, and 1.25% NAA). No terminal cuttings treated with auxin powder rooted. Generally, the rooting of cuttings was not improved by auxin powder.

Table 7.3. Rooting of koa cuttings treated with IBA and NAA solutions.

Treatment (w/v)		Stem cuttings (24 samples)		Terminal cuttings (12 samples)	
		# of rooted cuttings	Rooting frequency (%)	# of rooted cuttings	Rooting frequency (%)
IBA	0.25	2	8.3	1	8.3
	0.50	0	0.0	0	0.0
	0.75	0	0.0	0	0.0
	1.00	0	0.0	0	0.0
	1.25	0	0.0	0	0.0
	1.50	0	0.0	0	0.0
NAA	0.25	0	0.0	0	0.0
	0.50	0	0.0	0	0.0
	0.75	0	0.0	3	25.0
	1.00	0	0.0	2	16.7
	1.25	0	0.0	2	16.7
	1.50	0	0.0	0	0.0
CK 1 (water)		0	0.0	0	0.0
CK 2 (50% ethanol)		0	0.0	0	0.0

Table 7.4. Rooting of koa cuttings treated with IBA and NAA powder.

Treatment (%, w/w)		Stem cutting (12 samples)		Terminal cutting (12 samples)	
		# of rooted cuttings	Rooting frequency (%)	# of rooted cuttings	Rooting frequency (%)
IBA	0.25	0	0.0	0	0.0
	1.25	0	0.0	0	0.0
	2.25	2	16.7	0	0.0
	3.25	1	8.3	0	0.0
	4.25	0	0.0	0	0.0
	5.25	0	0.0	0	0.0
NAA	0.25	0	0.0	0	0.0
	1.25	1	8.3	0	0.0
	2.25	0	0.0	0	0.0
	3.25	0	0.0	0	0.0
	4.25	0	0.0	0	0.0
	5.25	0	0.0	0	0.0
CK 1 (water)		0	0.0	0	0.0
CK 2 (50% ethanol)		0	0.0	0	0.0

Effects of auxin, cytokinin, and synergic effect of cytokinin and auxin

Experiment 5 (Table 7.5) using IAA (acid form), NAA (acid form), IBA (salt and acid form), and 6-BA was conducted on cuttings from one tree of family 6HO1-4 at transitional stage. Rooting failure of cuttings had been observed as the result of leaf shedding. Thus auxin or cytokinin solutions in this experiment were sprayed on the leaves of cuttings before insertion. The results were more encouraging.

Table 7.5. Effects of foliage application of 4 growth regulators on rooting and leaf retention of cuttings from a tree of family 6HO1-4.†

Treatment (ppm)		# of rooted cuttings	Rooting frequency (%)	# of cuttings keeping the leaf	Leaf retention (%)
IAA (acid	10	3	25.0	3	25.0
form)	100	7	58.3	6	50.0
	1000	4	33.3	2	16.7
NAA(acid	10	1	8.3	2	16.7
form)	100	2	16.7	2	16.7
	1000	3	25.0	3	25.0
IBA (acid	10	5	41.7	6	50.0
form)	100	4	33.3	5	41.7
	1000	1	8.3	3	25.0
IBA (salt	10	0	0.0	0	0.0
form)	100	6	50.0	6	50.0
	1000	1	8.3	1	8.3
6-BA	10	5	41.7	9	75.0
	100	2	16.7	9	75.0
	1000	1	8.3	8	66.7
CK 1 (water)		3	16.7	3	16.7
CK 2 (50% ethanol)		1	5.6	1	5.6

†: All treatments had 12 cuttings, except CKs, which had 18 cuttings.

Both auxin and cytokinin showed some effects on rooting and leaf retention. Sprays of 100 ppm IAA and IBA (salt form), and 10 ppm IBA (acid form) greatly promoted the rooting of cuttings (58.3, 50.0, and 41.7% of three treatments vs. 16.7 and

5.6% of the two controls). All three concentrations of 6-BA increased leaf retention of cuttings, but high concentrations (100 and 1000 ppm) seemed to inhibit the rooting of cuttings. In follow-up trials auxin sprays neither increased leaf retention nor increased the rooting of cuttings from other trees. Only sprays of 6-BA had some positive effects on leaf retention (data not presented).

Experiment 6 was set up to test the effects of 6-BA and NAA on leaf retention and rooting of cuttings in more koa families (Table 7.6). Stem cuttings from trees of 4 families were first dipped into 1000 ppm IBA solution, and then sprayed with 0, 10, 50, and 100 ppm of 6-BA, and 0 and 500 ppm of NAA solutions.

Sprays of 10 to 100 ppm of 6-BA delayed leaf shedding. The delay was more obvious in first two weeks in some concentrations of 6-BA. Leaf retention was extended up to 5 weeks in family 1MN1-6. However, most families had only a delay of 2 weeks. The spray of NAA alone did delay leaf shedding. It indeed counteracted the effects of 6-BA after 3 weeks under mist. The delayed leaf shedding, although was not significant after 5 weeks of insertion, led to the rooting of a few cuttings. The cuttings of family 1MN1-6 gave the best rooting frequencies after sprays of 50 and 100 ppm 6-BA, (17.7 and 22.5 rooting percent of the treatments vs. 0% of the control). All but a few of the cuttings that rooted were from 6-BA treatments that were not combined with NAA.

Table 7.6. Effects of foliage application of 6-BA and NAA on leaf shedding of koa cuttings.†

Family	Treatment		Mean of cuttings shedding leaves after					# of cutting
	6-BA (ppm)	NAA (ppm)	1 wk.	2 wks	3 wks.	4 wks.	5 wks.	rooted
2W2-2	0	0	11	11.7	11.7	12.0	12.0	0.0
	10	0	6.3	10.3	11.3	11.7	12.0	0.0
	50	0	7.0	10.0	10.7	11.0	11.7	0.0
	100	0	4.0	8.3	10.0	10.7	11.3	0.0
	0	400	9.0	11.3	12.0	12.0	12.0	0.0
	10	400	4.3	11.0	12.0	12.0	12.0	0.0
	50	400	2.7	7.3	8.7	10.3	11.7	0.0
	100	400	6.0	8.3	10.0	10.7	11.3	0.0
KOKEEL2	0	0	6.7	9.3	10.7	10.3	11.3	0.0
	10	0	3.3	9.7	10.7	11.0	11.3	0.7
	50	0	0.7	4.7	7.3	9.0	9.3	1.0
	100	0	5.3	8.3	10.0	10.0	10.7	0.3
	0	400	7.7	11.0	11.3	12.0	12.0	0.0
	10	400	4.3	10.7	11.7	11.7	12.0	0.0
	50	400	0.7	10.0	10.7	12.0	12.0	0.0
	100	400	4.0	7.7	9.7	11.7	12.0	0.0
1MN1-6	0	0	0.3	4.3	8.3	10.0	11.0	0.0
	10	0	0.3	2.7	5.7	7.0	8.0	0.7
	50	0	0.3	2.0	2.0	3.0	6.7	2.7
	100	0	0.3	0.7	0.7	2.7	4.7	2.0
	0	400	0.7	5.3	10.3	11.3	11.3	0.0
	10	400	1.0	8.0	9.3	10.7	11.3	0.3
	50	400	0.0	0.7	2.7	6.0	10.0	0.0
	100	400	0.3	0.3	3.0	5.0	7.0	1.3
93-130-1	0	0	6.3	9.0	10.7	11.3	12.0	0.0
	10	0	2.0	5.7	10.7	11.0	11.3	0.0
	50	0	0.7	4.0	6.7	7.3	7.7	0.7
	100	0	0.3	4.7	6.3	8.0	8.7	0.3
	0	400	3.7	9.0	11.3	11.3	12.0	0.0
	10	400	2.3	8.0	10.0	11.3	11.3	0.0
	50	400	0.7	5.0	7.0	8.7	9.3	0.0
	100	400	3.0	5.3	7.7	10.3	10.3	0.0

†: All numbers are the average of three replications.

Etiolation

In Experiment 7, all 3 trees treated with small cages produced etiolated shoots. No cuttings from these shoots rooted. However, the etiolated cuttings survived much longer than the control cuttings (cuttings without etiolation). Etiolated cuttings survived as long as 6 weeks without leaf shedding, while the controls quickly died in 10 days of insertion (data not shown).

Only 2 of 3 trees covered with the big cages produced new shoots after 1 week, and those new shoots as well as old branches did not grow well in the cages. Original and new leaves shed off the branches followed by the death of the branches. It was apparent that in contrast to *Leucaena* hybrids, koa trees did not tolerate heavy shading, and new growth could not persist in dark cages. A few etiolated cuttings were obtained for rooting experiments after three weeks. All cuttings were sprayed with 10 ppm 6-BA prior to insertion to the medium to prevent leaf shedding.

The results (Table 7.7) showed that rooting was improved by the treatments of etiolation on the tree of 2W2-2. Successful rooting was achieved for 31.6 and 16.7% of cuttings treated with etiolation + Velcro belts and etiolation respectively, whereas no control cuttings rooted. Only 1 of 32 cuttings of 5F45P4 rooted, but etiolated cuttings did survive much longer than the control ones (data no shown).

Table 7.7. Rooting frequencies of etiolated single-node cuttings without auxin treatments after 6 weeks.

Family	Treatment	# of cuttings	# of rooted cuttings	Rooting frequencies (%)
2W2-2	Etiolation	18	3	16.7
	Etiolation+Velcro	19	6	31.6
	Control	18	0	0.0
5F45P4	Etiolation	18	0	0.0
	Etiolation+Velcro	14	1	7.1
	Control	21	0	0.0

Etiolation: Cuttings developed under 3 weeks of etiolation and 1 week of greening period. Etiolation + Velcro: Cuttings developed under 3 weeks of etiolation and 1 week of Velcro belts under sun.

Rooting of cuttings from mature trees

Experiment 8 was conducted in 3 mature trees (2PK2-1) from Maunawili and gave no rooted cuttings. All cuttings died after rapid leaf shedding, and no sign of rooting was found.

Experiment 9 (Table 7.8) using cuttings from a mature tree at Hamakua yielded very different results. The age of tree was 6 years and had started to set pods. Cuttings were treated with IBA (quick dips) or the combinations of IBA and 6-BA (sprays on leaves of cuttings). In contrast to cuttings from the tree of 2PK2-1 of Maunawili, cuttings from the tree of 6KMC1-5 of Hamakua responded to 6-BA and IBA very well. Rooting was found in most treatments except 0.75 and 1.00% IBA, and rooting frequencies ranged from 25 to 33.3% in IBA treatments. The spray of 6-BA did not significantly increase leaf retention of the cuttings but seemed to increase rooting frequencies (ranging from 8.3 to 41.7%). No cuttings rooted in control 1 (water), but there were 8.3% cuttings rooting in control 2 (50% ethanol).

Rooted cuttings sprayed with 100 ppm 6-BA solution had poorer root systems than the cuttings without the spray. Root qualities of cuttings treated with IBA are shown in Figure 7.2.

Table 7.8. Effects of growth regulators on cuttings from one mature koa tree 6KMC1-5.

Treatment		# of cuttings retaining the leaves	% of cuttings retaining the leaves	# cuttings rooted	Rooting frequencies (%)
IBA (%, w/v)	0.25	6	50.0	4	33.3
	0.50	4	33.3	3	25.0
	0.75	5	41.7	0	0.0
	1.00	2	16.7	0	0.0
	1.25	4	33.3	3	25.0
	1.50	3	25.0	3	25.0
IBA (%, w/v) + 6-BA (100 ppm)	0.25	7	58.3	3	25.0
	0.50	6	50.0	5	41.7
	0.75	2	16.7	5	41.7
	1.00	5	41.7	4	33.3
	1.25	4	33.3	1	8.3
	1.50	3	25.0	2	16.7
CK1	water	4	33.3	0	0.0
CK2	50% ethanol	6	50.0	1	8.3

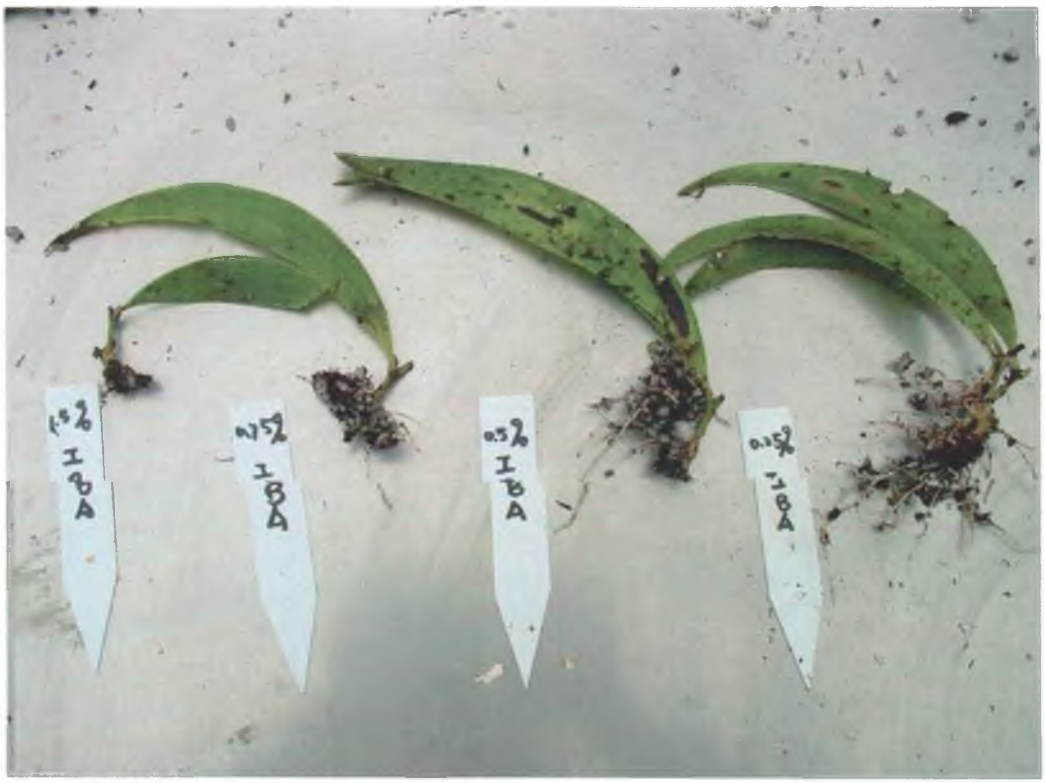

Figure 7.2. Phyllodinous cuttings of tree 6KMC1-5 rooted after auxin treatments.

Discussion

Results of the study indicate that it is possible to clone koa trees at juvenile stage. In our other cuttings operations, cuttings from vigorous juvenile seedlings usually rooted very well without the application of growth regulators (data not presented). There was variation in root ability among koa families as in many other woody species do. Such variations have been quantified as done in other forest species (Foster, 1990; Borralho and Wilson, 1994). However, due to the limited number of cuttings that could be taken from small koa seedlings, and their bad regrowth after pruning, such work became very difficult and was left unfulfilled.

Rooting potential of koa at juvenile stage decreased very quickly upon aging, as found in other *Acacia* species such as *A. mangium* and *A. auriculiformis* (Ahmad, 1991; Simsiri, 1991). Ahmad (1991) suggested that rapid decline of rooting frequency with increasing ages in *A. mangium* was possibly due to the thickening of sclerenchymatous cells. This might be true in koa too, but no single factor can fully explain failure of koa cuttings from mature trees. Koa trees tended to lose rooting ability even before they reach transitional stage, and most trees lost rooting ability completely at transitional and mature stages. Rooting ability of trees could be remained by using subclones of the trees, which were still at juvenile stage, as seen in Experiment 2. Since rooting ability of subclones also declines quickly, several cycles of cloning using subclones are expected if a large amount of clones are needed.

Cuttings of most koa trees entering transitional and mature stags did not respond to the application of rooting compounds such as NAA and IBA in a wide range of concentrations. The results were identical to those of Skolmen's study, where no

phyllodinous cuttings rooted after being treated with various concentrations of IBA and hydroponic solutions (Skolmen, 1977). Rooting of many woody species including *Acacia* species can be improved by rooting compounds such as NAA and IBA. It seems that it is not the case in koa.

Not all trees at transitional and mature stages failed to respond to growth regulators. Two trees, both of Hawaii Island origins, rooted well after being treated with growth regulators at transitional or mature stage. Koa populations from Hawaii Island are distinctive from those from other islands. They differ in tree form, phyllode shape, seed characters, and the speed of phyllode development (Sun, 1996; Daehler et al., 1999). As shown in this study, they might also differ in rooting ability from populations of other islands, but present data are too limited to permit a definitive conclusion.

Cuttings' failure to respond to rooting compounds seemed associated with leaf shedding of the cuttings, as it was evident that leaf shedding always preceded rooting failure. The presence of leaf on cuttings is very important for root initiation in many species (Hartmann and Kester, 2002). One such example is avocado, where earlier leaf shedding accompanies the difficulty of rooting in some varieties, and easy-to-root varieties have long leaf retention (Raviv and Reuveni, 1980).

The foliage application of a cytokinin 6-BA could delay leaf shedding up to 2 weeks in most studied koa families. However, such delay was apparently not long enough for root initiation. There was some evidence that the application of 6-BA could inhibit root initiation and development. The effects of 6-BA and NAA on leaf shedding delay were reported by Raviv and Reuveni (1984) for difficult-to-root avocado varieties. The authors found that 6-BA and NAA could delay leaf shedding and increase rooting

frequency of cuttings significantly. In avocado, 6-BA and NAA had different roles in leaf shedding delay. The 6-BA deferred the degradation of leaf chlorophyll, and both 6-BA and NAA delayed petiole abscission. Great increases in rooting frequency only occurred under the synergistic effect of 6-BA and NAA (Raviv and Reuveni, 1984). In my study, only 6-BA could delay leaf shedding, and no synergistic effect was found. Unlike in avocado, where the delay of leaf shedding caused by 6-BA and NAA could last 6 to 7 weeks, the delay of leaf shedding in koa was about 2 weeks only, a period not long enough for root initiation. Therefore a more effective technique to prevent leaf shedding appears essential for the application of rooting compounds.

One possible measure to delay leaf shedding and increase rooting ability of koa cuttings is etiolation. Etiolated cuttings of some koa families rooted without the assistance of rooting compounds. All etiolated cuttings kept their leaves and survived much longer than those grown under sun (40 days vs. 10days). Such cuttings have a great potential to root if they are treated with proper rooting compounds.

More techniques of etiolation treatment are needed to obtain quality etiolated cuttings. Etiolating an entire pruned koa tree may be feasible when the tree is still small, but it is totally unrealistic for big trees. Moreover, unlike other species like *Leucaena*, koa is extremely intolerant to heavy pruning and etiolation. In my study, a small cage was made to cover a single pruned branch, and quality etiolated cuttings were obtained. Such cages can also be used in big or wild trees if lower branches are available.

Conclusions

Koa stem cuttings rooted well at juvenile stage. Significant variations in rooting ability were observed among families tested. Rooting ability declined quickly as the age of trees increased. Most trees lost rooting ability at transitional stage. Cuttings failed to respond to the auxin treatments at transitional stage. All failures of rooting were accompanied by leaf shedding. The sprays of 10 to 100 ppm of a cytokinin 6-BA delayed leaf shedding up to 4 weeks in some families. However, such delays were not long enough for cuttings to initiate roots. Etiolation treatments on young shoots at transitional stage greatly increased the surviving period of cuttings, and led to increased rooting frequencies in one family. The etiolation treatments showed a great potential for future rooting experiments. Cutting of most families at mature stage also failed to root with or without auxin treatments. Only cuttings of one family originating from the island of Hawaii responded well to auxin treatments, and a moderate portion of cuttings rooted after the treatments. Another Hawaii-origin family also responded to auxin well at transitional stage, suggesting that some families originating from the island of Hawaii might have a unique rooting ability.

CHAPTER 8. MORTALITY OF KOA AND RESISTANCE TO KOA WILT

Introduction

Koa is one of the most prestigious forest species in Hawaii, due to its ecological, economic, and cultural importance. The area of koa forests has declined dramatically, as a result of historic deforestation. Progeny trials of koa were initiated by Dr. Brewbaker, following seed collections from healthy trees in the 1960's and 70's. Progeny trials were designed to evaluate the performance of koa progenies from major Hawaiian Islands, to quantify the genetic variability among koa germplasm, and to select genetically superior genotypes with characters of fast growth, straight bole, and high resistance to insects and diseases like koa wilt.

Progeny trials were started at the Hamakua Research Station on the island of Hawaii in 1991, with later trials at the Maunawili Research Station (HARC) on the Island of Oahu. Prior to that, only small-scale trials were made by USDA Forest Service, and no genetic variations were quantified (Skolmen, et al., 1991). Each UH progeny trial included around 50 half-sib families (including some composite families) from 4 major islands. A randomized complete block design with augmented entries was used in all trials. Data on early growth, phyllode development, and tree form were recorded and analyzed. Early data of the trials revealed that koa is a fast-growing species under careful management. Remarkable genetic variations in growth, tree form, and phyllode development were found (Sun, 1996). Most families (90%) turned out to be inferior, and about 10% of the families were selected for further improvement (Brewbaker, 1996).

A clear scenario has emerged of the infection of koa wilt disease caused by *Fusarium oxysporum* f. sp. *koae*. For example, in the earliest trial SET 91-1, only 72 trees were left out of total 1020 initial trees with a survival rate of 7.1% at the age of 12 years. Most death of trees is believed caused by this disease (Brewbaker, personal comm.). Koa wilt disease is a vascular disease invading trees from their root systems. The disease is characterized by inconspicuous symptoms in early infection, rapid tree death, and rare recovery of infected trees (personal observation). Although other factors might contribute to koa mortality, such as water deficiency, nutrition depletion, and various environmental stresses, the main cause is believed to be the wilt disease.

The objectives of this study were to evaluate koa family tolerance to koa wilt disease by studying survival rates, and to conduct family selection based on survival data. The survival rate of the family was expected to be a good index weighing resistance or tolerance to the disease, since only those trees with some resistance could survive.

Methods and Materials

Trials and field management

Table 8.1 is a summary of the 15 trials. The first trial was planted in 1991, and since then one or two trials were conducted annually. A total of 593 families (including duplicates) or 376 families (excluding duplicates) were involved in all trials. Most of them were half-sib families or composite seeds collected from healthy trees on major Hawaiian Islands. Some of them were seeds of advanced trees grown at Hamakua. The trials usually employed an augmented randomized complete block design with two replications. Ten individual trees were spaced at 1.5 x 1 m in two-row plots.

The sites of trial were well prepared before planting. Since 1995, drip irrigation has been used in the first year of planting to assist early establishment. In recent trials, an electric fence was used to keep wild pigs out. All trials were subjected to standard field management including weed control and fertilizing. Detailed information on nursery management and seed sources can be found in Sun's (1996) work.

Monthly and annual rainfall from 1991 to 2003 at Hamakua station is presented in Table 8.2.

Table 8.1. Summary of koa trials at the Hamakua Research Station, Hawaii Island.

SET number	No. of families	Purpose of trial	Experimental design
SET 91-1	48	Progeny test	AugRCB, 2 reps
SET 92-2	5	Spacing trial	SplitPlot, 3 reps, +/leucaena
SET 93-1	15	Progeny trial	AugRCB, 3 reps
SET 94-1	46	Progeny trial	AugRCB, 2 reps
SET 95-1	58	Progeny trial	AugRCB, 2 reps
SET 96-1	59	Progeny trial	AugRCB, 2 reps
SET 96-2	8	Demonstration	CRD, 20 tree plots
SET 97-1	80	Progeny trial	AugRCB, 2 reps
SET 98-1	27	Progeny trial	AugRCB, 2 reps
SET 99-1	66	Progeny trial	AugRCB, 2 reps
SET 99-2	15	Seed orchard	RCB, 13 reps, 1-tree plots
SET 00-1	47	Progeny trial	AugRCB, 2 reps
SET 01-1	50	Progeny trial	AugRCB, 2 reps
SET 02-2	45	Progeny trial	RCB, 2 reps
SET 02-3	24	Seed orchard	CRD, 10 reps, single tree plot

Table 8.2. Monthly and annual rainfalls (inches) from 1991 to 2003 at Hamakua station.

Year	Month												Annual
	1	2	3	4	5	6	7	8	9	10	11	12	
1991	3.48	5.67	43.18	5.20	7.87	4.62	3.94	13.47	3.68	1.10	0.52	8.99	101.72
1992	13.20	7.60	2.17	5.46	11.33	4.15	9.74	4.60	19.66	3.24	16.43	10.57	108.15
1993	3.81	0.83	15.38	9.67	10.61	4.06	12.65	7.78	5.44	10.69	19.37	11.52	111.81
1994	18.52	7.21	23.52	15.72	2.69	9.28	6.20	3.72	6.95	1.17	12.73	3.81	111.52
1995	11.12	2.34	5.96	16.22	11.83	3.40	5.65	6.43	1.67	2.70	0.30	1.35	68.97
1996	5.04	33.57	11.02	26.00	3.60	2.26	5.66	1.49	1.24	0.51	14.89	10.57	115.85
1997	9.13	9.63	8.07	2.31	12.04	10.54	11.50	2.40	4.95	2.00	29.88	12.89	115.34
1998	1.32	5.19	6.99	28.71	17.55	3.71	6.13	12.97	11.26	5.57	9.23	3.59	112.22
1999	11.28	13.88	25.50	4.36	0.19	1.25	1.04	2.85	3.79	1.94	9.94	21.35	97.37
2000	17.35	0.19	2.91	8.10	1.35	1.72	1.48	7.59	2.87	10.32	7.90	0.25	62.03
2001	0.52	15.30	5.56	5.72	1.83	1.82	2.91	6.79	4.06	14.41	11.81	12.99	83.72
2002	10.52	32.23	23.11	3.77	2.32	1.72	8.70	3.17	3.29	3.48	2.89	8.06	103.26
2003	4.92	17.55	1.61	4.52	0.55	2.44	9.15	--	--	--	--	--	--

Data collection and analysis

The trials were studied periodically, and the survival status of each tree (dead, dying, or missing) was recorded. Only trees marked as "living" were counted in survival rates, and no trees marked as "dying" were counted. Most data analyzed were 3- or 5-year survival data, since at these ages the survival rates of families were highly differentiated, which is considered suitable for family selection.

Survival rates were first calculated for families in each block of a specific trial. Arithmetic means of the blocks and families were then used to calculate the survival rates of the family and trial. The survival rate of each family in each block was calculated as the percent of surviving trees out of total planted.

ANOVA was conducted on replicated families to test the effects of family and island on survival rates. The ANOVA was conducted on the SAS® system using PROC ANOVA procedure. Correlation coefficients between family survival rates and DBHs (diameter at breast height) at a specific age were conducted by PROC CORR.

Results

Overall trend of survival

The survival rates of each trial and the average survival rate of all trials at given ages are summarized in Table 8.3. Average survival rates of the trials steadily decreased over the ages. During the first four years of planting, the survival rates varied widely from trial to trial. Especially at the age of one year, the overall survival rates of the trials ranged from 54.4% (SET 01-1) to 96.0% (SET 95-1). Several factors influenced this early mortality including genetic inferiority, drought in summers, late planting dates, animal grazing, and the main factor—wilt disease. Variation of overall survival rates among the trials tended to become smaller as the ages of trials increased. At the age of 5 years, all the trials had overall survival rates ranging from 29.7 to 42.0%, a range much smaller than that of age one year. After 7 years of planting, the overall survival rates of all the trials were very close, ranging from 22.5 to 27.4%. A rough pattern of seedling losses is drawn in Figure 8.1. Seedlings were lost sharply from age 1 to 6, and there was a platform from age 6 to 9.

Table 8. 3. Individual and average survival rates of the trials of Hamakua at different ages.

SET no.	# of family	Date of planting	Overall survival rate (%), at the age (years) of											
			1	2	3	4	5	6	7	8	9	10	11	12
91-1	48	May 23	88.2	77.0	46.5	43.1	40.4	-	-	-	16.2	8.4	7.8	7.2
93-1	15	May 27	67.5	58.6	55.4	49.8	-	-	24.0	22.0	18.1			
94-1	45	May 26	68.0	-	-	-	-	-	22.8	19.7	17.9			
95-1	53	May 19	96.0	-	-	74.7	39.9	27.0	27.4	24.5				
96-1	59	May 23	-	-	37.7	28.1	28.8	25.4	22.5					
96-2	8	Mar 15	-	-	73.3	-	42.0							
97-1	80	May 21	72.4	-	50.1	43.0	38.6	32.2						
98-1	27	Aug 13	81.5	-	41.3	30.7	29.7							
99-1	66	July 9	58.7	47.9	40.0	34.5								
00-1	47	Aug 2	76.1	54.5	46.3									
01-1	48	May 16	54.4	41.5										
Avg.			73.6	61.8	48.8	43.4	36.6	28.2	24.2	22.1	17.4	8.4	7.8	7.2

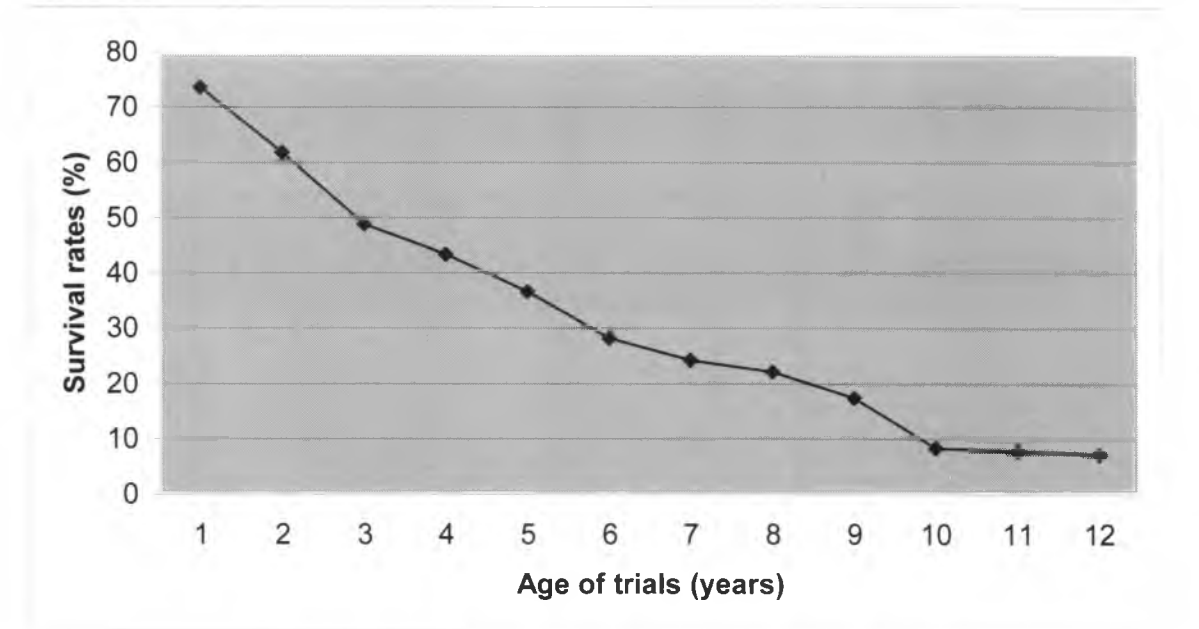

Figure 8.1. Overall survival rates of the trials over the years.

Variation of survival rates among families and islands

Survival rates of koa families at the age of 3 years or in some case at the age of 5 years were calculated to examine koa family's resistance to wilt disease. Average survival rates of all the families are presented in Table 8.4 and 8.5. Koa families showed

great differences in survival in all trials. Some outstanding families had high survival rates at age 3 or 5, while some families were completely lost.

Since those families were from four major islands, which have unique geographical characters, families from different islands might have different resistance to the disease. Calculated effects of islands are presented in Table 8.6. Average survival rates of families from Kauai were the lowest compared to those from Oahu, Maui, and Hawaii in SET 00-1, 99-1, and 97-1, but it had similar rates in SET 95-1. Families from Maui had high survival rates in all trials. Families from Oahu generally survived well except in SET 93-1. Families from Hawaii survived well in most trials (SET 00-1, 97-1, 94-1, and 93-1), but did only fairly in SET 99-1 and 95-1. Different performance of families from the same island at different trials might be caused by non-random representations or small numbers of the families. For example, those families of Kauai, which survived badly in SET 00-1 and 99-1, were small seed collections made by HARC. If such biasing factors were excluded, it seemed that there was no significant effect of island on surviving.

Table 8. 4. Mean family survival rates (%) of SET 95-1 and 97-1 at the age of 5 or 3 years.

SET 95-1(age 5)				SET 97-1 (age 3)			
Family	SURV.	Family	SURV.	Family	SURV.	Family	SURV.
1PP1C	55	2WT1-4A	45	1MA1-1	75	6KEA1-4	70
1PT1C	60	5CP1-2	50	1MA1-2(97)	65	6KEA1-5	55
1WM1C	20	5CP1-3	15	1MC1-1	70	6KEA1-6	55
2AA1-5	25	5CP1-5	90	1MN1-5	60	6KEA1-8	40
2HL1-1	20	5GUL1C	50	1MN1-6	40	6KEA1-9	10
2KH2-1	60	5KH1C	30	1NA1-1	50	6LA1-1	40
2KU1C3	5	5KO1-1	20	1WL1-2	45	6LA1-4	50
2KW1-2	80	5KP1C	40	2HA1C(91S)	65	6UM1-1	60
2KW1-4	65	5MA1-2	20	2KP2-1(94)	25	6UM2-2	60
2KW2-1	15	5SM1-2	60	2KU1-1(91S)	25	6UM2-3	60
2KW2-2	40	6HAM1C	35	2KU2-1(91S)	25	6UM2-4	60
2KW3-1	40	6HK1C	25	2KW2-1(95)	25	6UM2-5	35
2MI2-1	40	6HK2C	5	2KW2-2(95)	15	6UM3-1	75
2NU2-1	65	6KH1C	40	2MA2-1(94)	95	6UM3-2	75
2PH2-2	60	6KH2C	40	2PH2-2(94)	15	6UM4-1	45
2PH2-3	50	6KK1C	20	2PH2-3(95)	45	1LA1C(97) †	30
2PK1-2	25	6KM1C	35	6HO1-1	75	1MO1-1	20
2PK1-3	20	6KP1C	40	6HO1-10	45	1N1-5(97S)	70
2PK2-1	30	6KUC	0	6HO1-11	65	2KU1-1(121-6)	40
2PK2-1B	45	6LP2C	20	6HO1-2	75	2KU1C3	0
2PK2-2	35	6ML1C	40	6HO1-3	80	2ML1-1(91X223-1)	30
2PK3-1	80	6ML2C	55	6HO1-4	50	2ML1-9(91)	70
2PK3-1B	15	6MLR5-1	20	6HO1-5	45	2W2-2(94)	40
2PO1-2	40	6OF1C	45	6HO1-6	75	5KGB1-1	30
2W2-2	85	6PUU1C	55	6HO1-9	40	6HO1-7	70
2W3-4	80	6SAD1C	20	6HO2-1	85	6HO1-8	70
2W4-2	25	6WV1-1	55	6HO3-2	80	6HO2-2	90
2W4-3	45	6WV1-2	40	6HO3-3	55	6HO3-1	70
				6HO3-4	50	6HO4-3	0
				6HO4-1	65	6KA1-1	10
				6KE1-1	45	6KEA1-13	50
				6KE1-2	55	6KEA1-7	40
				6KE1-3	55	6KL1C	30
				6KE1-4	55	6KU1-1(91S204-3)	20
				6KEA1-10	30	6KU1C3	0
				6KEA1-11	40	6PK1-2	30
				6KEA1-12	40	6PUU1-1	30
				6KEA1-14	85	6UM1-3	50
				6KEA1-2	40	6UM2-1	60
				6KEA1-3	50		

SURV: Mean survival percentage.

†: Below are unreplicated families.

Table 8. 5. Mean family survival rates (%) of SET 99-1 and 00-1 at the age of 3 years.

SET 99-1 (age 3)				SET 00-1 (age 3)			
Family	SURV.	Family	SURV.	Family	SURV.	Family	SURV.
1KAHANA C	40	6HAM2	50	1KAHANA-C	75	2PK2-1	40
1OP5	40	6KALO10	45	1OP1	40	2PK4	20
1OPR10	40	6KALO11C	85	1OP3	80	2PK5	10
1OPR2	40	6KALO8	65	1OP4	80	5DTF1	70
1OPR4	45	6KAPA4	30	1OP5	50	5F45-3	55
1OPR5	40	6KAPA5	75	1OPR1	85	5F45P2	70
1OPR8	40	6KAPA6	25	1OPR10	50	5F45P4	38
1OPR9	75	6KAPA7	70	1OPR2	45	6-HAM 1-1	50
2ANAHOLA1	15	6KLIC	30	1OPR4	35	6-HAM 1-3	80
2ANAHOLA2	10	6KLIC1	40	1OPR5	65	6HAM1	70
2HALELEA1	15	6KLIC5	40	1OPR6	50	6HAM2	40
2NONOU1	25	6KLIC7	15	1OPR7	60	6KAPA2	70
2NONOU2	15	6KLIC9	35	1OPR8	40	6KAPA3	85
2W4-5	30	1MA1†	30	1OPR9	60	6KAPA5	80
5F45P2	65	1OP1	60	2ANAHOLA1	10	6KAPA7	60
5F45P3	85	1OP2	30	2ANAHOLA2	0	6KAPA-C	22
5F45P4	40	1OP3	70	2HALELEA	0	6KLIC1	40
6HAM '91-124-8	40	1OP4	60	2HFRC	0	6KLIC2	28
6HAM '91-149-9	50	1OP6	60	2KOKEE1	20	6KLIC4	75
6HAM '91-154-5LE	40	1OPR1	90	2PAPA1	10	6KLIC6	55
6HAM '91-202-10	20	1OPR6	30	2PAPA2	20	6KLIC7	60
6HAM '91-206-3	50	1OPR7	70	2PAPA3	20	6KLIC8	75
6HAM '91-212-9	20	1PACP C	20	2PK1 (PK S. LUA)	10	6KLIC9	75
6HAM '91-218-9	55	2HFR C	0	2PK2	10		
6HAM '91-222-2	30	2PK2-1A	10				
6HAM '91-228-6	20	2PK2-1B	20				
6HAM '92-107-4	35	5CPI-1	0				
6HAM '92-204	15	6HAM1	60				
6HAM '92-303	25	6KLIC3	20				
6HAM '92-308-12	60	6KOIA2	20				
6HAM '93-302-12	20	6KOIA3	80				
6HAM '93-313-9	35	6KOIA1	50				
6HAM '94-107C	20	6KOIA5	30				

SURV: Mean survival percentage.

†: Below are unreplicated families.

Table 8.6. Island averages of tree survival rates (%) of 6 progeny tests.

Island	SET 93-1 (age 3)	SET 94-1 (age 7)	SET 95-1 (age 5)	SET 97-1 (age 3)	SET 99-1 (age 3)	SET 00-1 (age 3)	Overall
Oahu	39.6	--	45.0	57.9	47.7	58.2	49.7
Kauai	--	22.8	43.7	37.2	16.7	13.1	26.7
Maui	--	--	41.7	--	54.3	58.3	51.4
Hawaii	68.1	21.6	32.8	55.6	39.7	60.3	46.4

Analyses of variance of replicated families were conducted on two analysis models--with or without the effect of island. If the effect of island was not considered, the differences of survival rates of families were significant in all trials (ANOVA was not presented in tables). The distributions of family survival rates are presented in Figure 8.2. Survival rates of families varied widely within the trials, and were close to a normal distribution. Such differentiation is expected to be an advantage for family selection.

If the effect of island was considered, the differences of survival rates among the islands were found significant in 3 sets of trial (SET 97-1, 99-1, and 00-1), but not significant in SET 95-1; whereas the differences among the families within the islands were still significant in 3 sets of trials (SET 95-1, 97-1, and 99-1), but not significant in SET 00-1 (Table 8.7).

Table 8.7. ANOV of survival rates of 4 sets of trials including island effects.

	SET 95-1 (age 5)		SET 97-1 (age 3)		SET 99-1 (age 3)		SET 00-1 (age 3)	
Source	DF	SG	DF	SG	DF	SG	DF	SG
Island	3	NS	2	**	3	***	3	***
Family (island)	51	**	52	*	42	**	43	NS
Rep	1	NS	1	NS	1	NS	1	NS
Error	54		54		45		46	
Total	109		109		91		93	

NS, *, **, and ***: Non-significant, significant at $p<0.05$, 0.01, and 0.001 respectively.

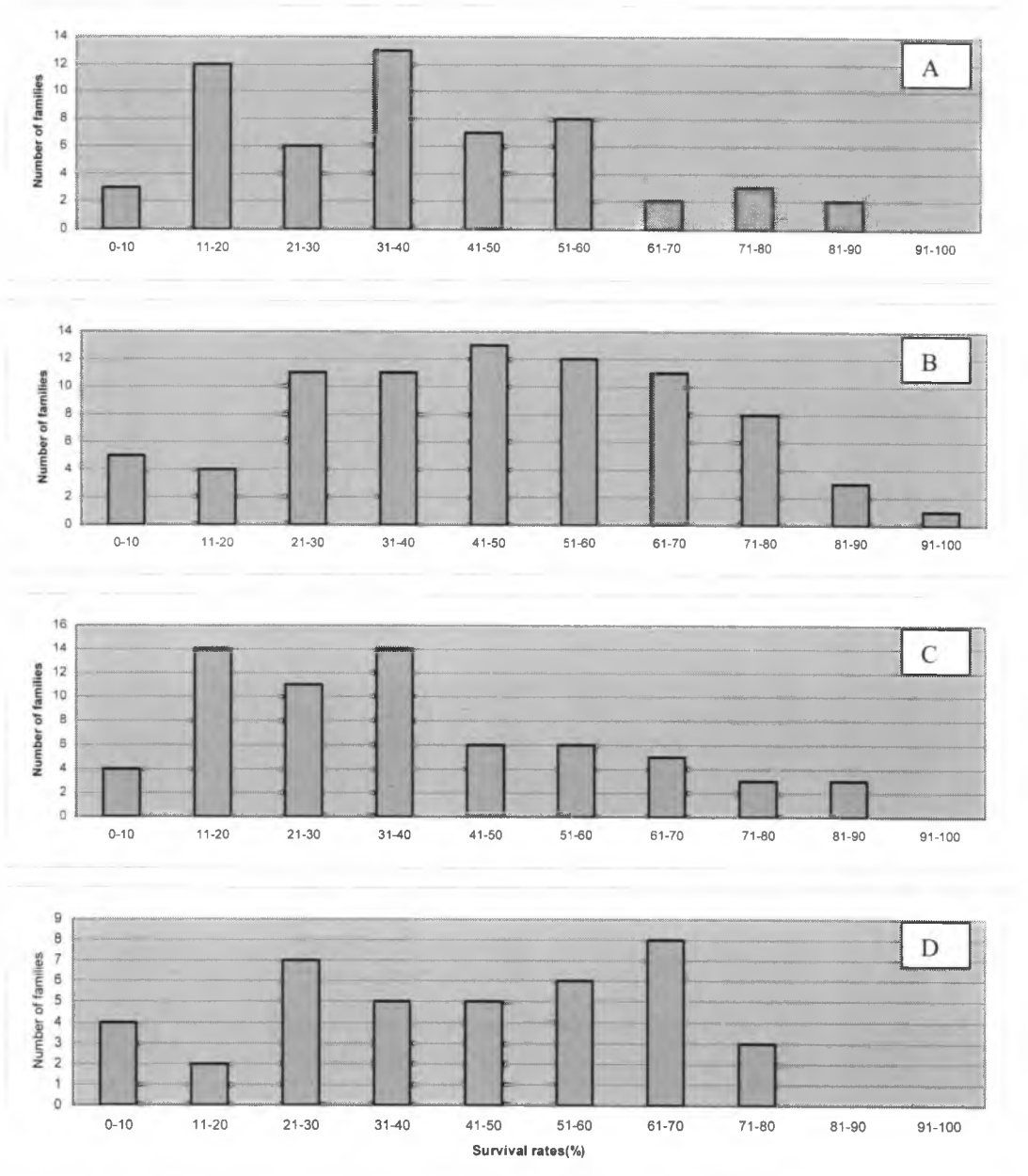

Figure 8.2. Distributions of family survival rates. A: SET 95-1 at the age of 5 years. B: SET 97-1 at the age of 3 years. C: SET 99-1 at the age of 3 years. D: SET 00-1 at the age of 3 years.

Effects of family survival rates among seed sources

All our koa seeds were collected from a wide range of seed sources, which have unique geographical natures. Koa populations displayed distinct characters of growth habit and morphology (Sun, 1996; Daehler et al., 1999). To determine if seeds from a specific location had better survival than seeds from other sources, the effects of seed sources were calculated. Here one seed source refers to all families from the same specific location. Since all our koa families were coded by the abbreviation of the locations where seeds were collected, families with the same location code were regarded from one seed source. Many seed sources were represented by only several families in each trial, thus no ANOV including the effect of seed sources and families was conducted.

Calculated average survival rates of seed sources are given in Table 8.8 and Table 8.9. In general, different seed sources displayed different survival rates. Some seed sources had consistent survival rates across the trials, while a few other seed sources had different survival rates across the trials. Such inconsistency might be caused by the small numbers of families representing the seed sources. Some excellent seed sources with a large number of families included Opaeula of Oahu, Honaunau, Kapa Pala, Keanakola, Umikoa Ranch, and Hamakua of Hawaii; Waimea Canyon Drive and Makaha of Kauai; M.P. We Field of Maui. Overall survival rates of those seed sources exceeded 50% at age 3 or 5. Typical bad seed sources included Puu Ka Pele and Anahola from Kauai. Families of Puu Ka Pele had overall survival rates of 9.0, 18.0, and 35.7% in three trials, and families of Anahola had survival rates of 5 and 12.5% in two trials. Seeds of

advanced generation from the Hamakua Research Station also had low survival rates (overall 34% at age 3).

Since the variation of survival rates within the seed source was also large, even a seed source was regarded "bad", there were still families with high survival rates from this source. For example, most families from Puu Ka Pele of Kauai had very low survival rates and grew very slowly (Sun, 1996), but one family of 2PK3-1 had a high survival rate of 80% at the age of 5 years. Vice versa, there were families with low survival rates in those seed sources, which were regarded "good". The identification of good seed sources might be more helpful for future seed collection than for family selection. The selection of families with high resistance to the disease should be more reasonably based on the performance of families than on that of seed sources.

Table 8.8. Average survival percentages of seed sources of SET 94-1, 95-1, and 97-1 at age 7, 5, or 3.

SET 94-1 (age 7)	SURV	# of families	SET 95-1 (age 5)	SURV	# of families	SET 97-1 (age 3)	SURV	# of families
2 Kumuwela Trial†	12.5	2	2 Kaaweiki	48.0	5	1 Manana	70.0	2
2 Makaha Ridge Road	25.0	6	2 Puu Hinahina	55.0	2	1 Manana Trail	50.0	2
2 Miololii Ridge Road	34.0	5	2 Puu Ka Pele	35.7	7	2 Kaaweiki	20.0	2
2 Puu Hinahina	26.2	4	2 Waimea Canyon	58.8	4	2 Kumuwela	25.0	2
2 Puu Ka Pele	5.0	3	5 Copp Road	51.7	3	2 Puu Hinahina	30.0	2
6 Kilauea Mil. Camp	25.7	7	6 Hakalau	15.0	2	6 Honaunau	63.2	14
6 Mauna Loa Road	18.3	9	6 Keahou	40.0	2	6 Keahou	52.5	4
						6 Keanakola	46.8	11
						6 Laupahoehoe	45.0	2
						6 Umikoa Ranch	58.8	8

†: Numbers before the names of seed sources indicate the islands where they came from. 1: Oahu. 2: Kauai. 5: Maui. 6: Hawaii.

Table 8.9. Average survival percentages of seed sources of SET 99-1 and 00-1 at age 3.

SET 99-1 (age 3)	SURV	# of families	SET 00-1 (age 3)	SURV	# of families
1 Opaeula (low elevation) †	46.7	6	1 Opaeula (low elevation)	62.5	4
2 Anahola	12.5	2	1 Opaeula (high elevation)	54.4	9
2 Nonou	20.0	2	2 Anahola	5.0	2
5 M.P. We Field	63.3	3	2 Papa	16.7	3
6 HAM advanced generation	33.4	16	2 Puu Ka Pele	18	5
6 Kalo	65.0	3	5 M.P. We Field	54.4	3
6 Kapa Pala	50.0	4	6 Hamakua	60.0	4
6 Koa for Kulani	32.0	5	6 Kapa Pala	63.4	5
			6 Kohalele Ranch	58.3	7

†: Numbers before the names of seed sources indicate the islands where they came from. 1: Oahu. 2: Kauai. 5: Maui. 6: Hawaii.

Family selection based on survival rates

The top10% of families in survival rate are presented in Table 8.10. Those selected families were from all four islands. Most families were concentrated in limited seed sources. For example, five selected families from Oahu were from one seed source, Opaeula (OP and OPR represent different elevations of Opaeula). For families from Kauai, two main seed sources were Milolii Ridge Road and Waimea Canyon Drive, along with families from other sources. Seed collections of Maui are limited both at UH and HARC, therefore only two families were selected. Selected families of Hawaii were mainly from three seed sources--Honaunau, Kapa Pala, and Keahou.

Table 8.10. Koa families with the highest survival rates.

Family	Rep1 (%)	Rep2 (%)	Mean (%)	SET	Age
1OP3	80	80	80	00-1	3
1OP4	90	70	80	00-1	3
1OPR1	80	90	85	00-1	3
1OPR1	90	90	90	99-1	3
1OPR9	80	70	75	99-1	3
2HA1C('91-222-2)	60	60	60	98-1	3
2KW1-2	100	60	80	95-1	4
2MA2-1(94)	90	100	95	97-1	4
2MI1-1	--	--	85	93-1	3
2ML1-1	70	50	60	94-1	7
2ML2-4	50	70	60	94-1	7
2NU2C(91-131-3)	80	60	70	98-1	3
2PK3-1	90	70	80	95-1	4
2W2-2	100	70	85	95-1	4
2W3-4	70	90	80	95-1	4
5CP1-5	100	80	90	95-1	4
5F45P3	100	70	85	99-1	3
6HAM 1-3	80	80	80	00-1	3
6HO1-3	90	70	80	97-1	4
6HO2-1	90	80	85	97-1	4
6HO2-2	--	90	90	97-1	4
6HO3-2	70	90	80	97-1	4
6KALO11C	80	90	85	99-1	3
6KAPA3	80	90	85	00-1	3
6KAPA5	80	80	80	00-1	3
6KAPA5	80	70	75	99-1	3
6KAPA7	90	50	70	99-1	3
6KEA1-14	80	90	85	97-1	4
6KEAC	--	--	71	93-1	3
6KMC1-5	60	40	50	94-1	7
6KUK1C	--	--	69	93-1	3
Composite, bulked '91 trial	50	80	65	98-1	3

Correlations of survival rate and plant growth

The characters of resistance to diseases, pests, and adverse environments are normally genetically independent of economic traits, such as straightness and growth rate in forest trees (Zobel and Talbert, 1984). This makes it possible to select families simultaneously with high resistance to diseases and pests, and with good economic traits. For koa family selection, a question could be raised that if the mortality of trees within families significantly affected the growth of the survived trees in the same plot due to less competition, or if the surviving ability could be negatively correlated with growth rate. The correlation analysis of family survival rate and mean family DBH was conducted to answer this question. The results of analysis in five trials are presented in Table 8.11. All correlation coefficients of survival rate and DBH were very small (0.068 to –0.160), and no significant correlations existed between family survival rate and mean family DBH. The results indicated no effect of dead trees on the growth of surviving ones, and the independent inheritance of resistance to the disease from tree growth rate.

Table 8.11. Correlations of survival rates and DBHs.

Set	Age	Correlation coefficients of survival rate and DBH	Probability
94-1	7	-0.052	0.6753
95-1	6	0.068	0.5175
97-1	4	-0.085	0.3388
99-1	4	-0.140	0.1711
00-1	3	-0.160	0.1420

Discussion

Continuing death of koa trees over the years is a stunning phenomenon in those progeny trials. Several causes of koa mortality are evident. It is a consensus that the main cause of death is koa wilt, caused by the pathogen *F. oxysporum* f. sp. *koae*. *Fusarium oxysporum* was first found and isolated from young koa seedlings with wilt symptoms (Gardner, 1980). More isolates of the pathogen including one from the Hamakua Research Station have been recovered from various koa populations with dieback symptoms. However, late work on inoculation of the pathogen back to healthy mature trees did not cause wilt symptoms immediately (Anderson et. al., 2002). *Fusarium oxysporum* is an asexual fungus that is normally endophytic in many plants but rarely pathogenic. However, some mutant strains are pathogenic, and cause a wide range of wilt diseases (Gordon and Peterson, 2002).

Many biotic and non-biotic factors such as environmental stresses may also contribute to koa wilt (Anderson et. al., 2002; Brewbaker, personal comm.). For example, high competition among trees at a high planting density (initially 1 x 1.5m spacing) might have facilitated the spread and infection of the pathogens. A study on natural koa stands found that the stands tend to remain a stable basal area of the trees, and as a result, some small trees die over the years, while the diameters of dominant trees increase (Fownes, 1996). It seems wise to increase spacing of trees, or to thin out trees after several years to alleviate competition pressure in future progeny trials.

Occasional drought at Hamakua might have also weakened koa trees and thus have facilitated the infection of the fungus. Although generally the Hamakua station received more than 100 inches of annual rainfall since 1991, drought occurred often in

the summers (Table 8.1). Koa is a species having only a shallow root system. Drought, even for short periods, would definitely cause stresses on koa trees and enhance disease infection.

Koa trees infected by wilt do not show symptoms until later stages of infection, unlike other forest diseases like rust, canker, or gall, whose symptoms are easily visible. No trees infected by the fungus appeared to recover from the disease. It was impossible to identify resistance by scoring disease symptoms. In contrast, we used survival rates of the families at certain ages as an index weighing resistance to the disease, apparently the only appropriate way to assess tolerance. The effectiveness of family selection based on survival rates should be further tested in the future by other methods, such as early screening of family resistance using artificial inoculation of *F. oxysporum*.

It appeared that early mortality data (in 3 to 5 years) were proper for analysis of resistance since they varied greatly among the families tested. Late survival data appeared to be impractical, because of high mortality levels, which could be as high as 90%.

Some caution should be taken when mortality data were used to interpret disease tolerance, since other factors might also contribute to early mortality. Some koa families were genetically inferior and had difficulty in establishing in our progeny trials. Especially as noted by Sun (1996), some round-seed families from Kauai generally grew slowly and tended to die in early years, which was nothing to do with wilt disease. Late planting, evidenced in trials of 1998, 1999, and 2000, also caused some mortality of seedlings due to difficult establishment of seedlings in hot summers (Brewbaker, personal comm.).

Wilt disease is extremely severe in koa as shown in our trials, and it poses an immediate threat to potential koa plantations and reforestations. Variation in resistance to the disease is suggested to have a strong genetic base, providing us an opportunity to select resistant families through long-term breeding programs.

Family selection based on island or seed source may not be highly effective due to variations within the islands and seed sources. The best families in terms of survival rate occurred on all four major islands and from many seed sources.

The inheritance of family survival rates was found to be independent of the growth rate in our study, as found in many forest species where disease resistance is independent of traits like tree form and wood qualities (Zobel and Talbert, 1984). Such a phenomenon makes it possible to select families with high adaptability and high growth rates simultaneously. In our progeny trials, some families with high survival rates had low growth rates or bad tree forms. Similarly, some families with high survival rates grew fast and had straight tree boles.

Conclusions

The mortality of koa progeny trials almost linearly increased with the age. Many factors contributed to death of koa trees including genetic inferiority of the families, diseases, drought, and animal grazing. The main factor causing high koa mortality was koa wilt disease, a disease damaging the vascular system of koa trees. Variation in resistance to this disease was found among families. Such genetically based variation in disease resistance should provide a good basis for family selection. No correlations were found between growth rate (DBH) and survival rate, indicating independent inheritance

of these two traits, an advantage to select families with high growth rate and high resistance to the disease.

CHAPTER 9. RANKING KOA FAMILIES USING PREDICTED BREEDING VALUES

Introduction

The purposes of progeny trials are to determine the variability of germplasm, to quantify the genetic parameters of germplasm, and to conduct selection on germplasm. Koa progeny trials were started by Dr. Brewbaker and students in 1991 at the UH Hamakua Research Station. Twelve half-sib progeny trials have been conducted at Hamakua, and some trials were duplicated at the Maunawili Research Station of HARC (Hawaii Agriculture Research Center) (Sun, 1996). Researchers in HARC also conducted progeny trials using their own germplasm (Dudley, personal comm.).

Genetic variability of koa in growth, tree form, and morphological characters has been evident in these trials (Sun, 1996; Daehler et al., 1999). Sun (1996) concluded that koa is a fast-growing species. Large variations in growth and survival rate, tree form, and phyllode development exist among half-sib families. Dudley (2003) reached the same conclusions from his trials in HARC. Variance components for height, DBH, and phyllode development were also estimated by Sun (1996), based on early growth data.

Family ranking is one of the main purposes of progeny trials and is the basis for selection. The selection itself can be either based on families or individual trees, and can be characterized as backward or forward. Backward selection means going back to the seeds of those elite mother trees or mother trees themselves by vegetative propagation. Forward selection conducts advanced progenies of selected trees (Hodge and White, 1992).

Ranking families can be problematic when the trials are located in several locations, and each trial is represented by different sets of families. The fixed effects of trials (in different locations or years) will be confounded with family means, which makes it difficult to compare families from different trials directly. Different experimental errors and variances of family means among the trials also cause different precisions of estimation of family effects (Cotterill et al., 1983; White and Hodge, 1988).

Many approaches have been developed to rank families in progeny trials (Cotterill et al., 1983; Hatcher et al., 1981). The simplest method is taking an arithmetic average of all family means, which are expressed as percents of overall average or as deviations from the mean of a specific trial (White and Hodge, 1989). The use of percentages or deviations from the mean minimizes the environmental effects among trials, but does not evaluate G x E interactions. This method works well in trials represented by the same families and with equal experimental precision. The methods of least square estimates and generalized least square estimates of family means can minimize the effects of unequal representation of families in all trials and unequal experimental precision (White and Hodge, 1989).

One approach to homogenize variances from different trials is to use averaged standard scores that express the family means in terms of standard deviation. A simple score can be provided by dividing the deviation of the family mean by the estimated standard deviation. A transformation was called "Z" score by Hatcher et al. (1981). The "Z" scores of the same family across all trials then can be averaged for family ranking.

Other methods of estimating average performance of families across progeny trials described by Cotterill et al. (1983) include log transformation, rank-score, site-

adjustment, standard site-adjustment, least squares, weighted least squares, and shrunken least squares. All the methods have some rationales to rank family means. Least square methods tend to be more accurate, since the variance of unbiased estimate of family effects is minimized based on linear models (Cotterill et al., 1983).

All the methods mentioned above treat family means as fixed effects. Other ranking approaches use predicted breeding values on a mixed model, reported by White and Hodge (1988) in application to forest trees. The approaches are called Best Linear Prediction (BLP) and Best Unbiased Linear Prediction (BLUP). The effects of site, trial, and population are treated as fixed effects, while the effect of family is treated as a random effect (breeding value). The fixed effects can be estimated by ordinary least squares in BLP or by weighted least squares in of BLUP. The breeding value is predicted by the correlation between the additive genetic effect of the family and the family mean.

BLP and BLUP were developed in animal science to predict the breeding values of parents (Henderson, 1974, 1975). White and Hodge (1988) first adopted BLP to slash-pine improvement programs. The breeding values predicted by BLP were used for parental ranking. The ranking result of BLP was comparable to that of standard scores, but BLP appeared superior to standard scores when there were many missing trees. Also, the parents present in a large number of more precise progeny trials tended to be selected by using BLP ranking. In contrast, those parents in few, less precise trials were selected by standard score method (White and Hodge, 1988). The authors also suggested that for most half-sib progeny trials, where error variances are large, ordinary least squares are accurate enough to estimate fixed effects (White and Hodge, 1988).

The predicted breeding value is also useful in the calculation of genetic gain of the selection (Hodge and White, 1992). Since the predicted breeding value better approximates the true genetic worth of a family, predicted genetic gain can be calculated by averaging the predicted breeding values of selected families.

Main purpose of koa progeny trials is to provide a basis for selection, which includes backward and forward selection. The selection of family itself must be based on family ranking. However, no serious family ranking has been conducted on those koa progeny trials. Family ranking for our koa trials is difficult. First, most trials are single site, each is represented by a completely different set of families, and no common control families have been planted. Therefore, genetic effect of families is highly confounded with the effect of trial. Second, high mortality after years of planting makes only the ranking of early growth effective.

In this study, by predicting breeding values of the families using BLP, families in different sets might be more comparable, since the effects of trials were minimized. Moreover, genetic gain could be predicted on the basis of predicated breeding values.

The objectives of this study were:

1) to apply the methods of BLP to predict the breeding values of koa families;
2) to calculate the breeding values of koa families;
3) to rank family performance using breeding values predicted by BLP;
4) to predict genetic gains of family selection.

Because of high mortality of the trials after years of planting, we made prediction of breeding values only at certain age when a reasonable number of trees were alive.

Materials and methods

The mortality of the koa trials continuously increased with the age (see Chapter 8), and after the age of 5 years the survival rates were so low that too few data of individual trees were available for genetic parameter estimation. Thus the data used in BLP were 4-year data. The prediction of breeding values was made only on DBH, since these were only data available at the age of 4 years. No predictions were made on traits such as height, wood volume, and tree form. To make families across different trials comparable, all predictions were made on the same age of 4 years. Four sets of trial--SET 91-1, 95-1, 97-1, and 98-1--with data available were selected for this study. SET 98-1 included seeds from an advanced generation, while the other three trials involved half-sib families from native koa trees. The methods of koa progeny trials have been described in Chapter 8 and will not be repeated in this chapter.

Statistical analysis

The following mixed, single-tree model was adopted for statistical analysis:

$y_{kilj}=\mu+ B_i +I_k + f_l+fb_{li} +w_{ilj}$

where

$y_{kil\,j}$= the observation of single of l^{th} family from k^{th} island in i^{th} block,

μ = the fixed general mean,

B_i = the fixed effect of i^{th} block,

I_k = the fixed effect of k^{th} island,

f_l = the random effect of l^{th} family,

$E(f_l) =0 \quad Var(f_l)=\sigma^2_f$

fb_{li}=the random interaction effect of l^{th} family and i^{th} block

$E(fb_{li}) = 0$ $Var(fb_{li}) = \sigma^2_{fb}$

w_{kilj} = the random tree error of j^{th} tree in il^{th} plot

$E(w_{kilj}) = 0$, $Var(w_{kilj}) = \sigma^2_w$, and the variances between all pairs of factors are assumed zero.

ANOVA based on single-tree observations was conducted on the SAS^R program using procedure "PROC GLM". Since the data were unbalanced, and the effects of families were nested in the effects of the islands, type III sum squares were used in F tests.

Predicting breeding value with Best Linear Predication (BLP)

The formula to predicting breeding values with Best Linear Predication (BLP) is written below as Formula 9.1. The detailed derivation of the formula was given by White and Hodge (1988).

$$\hat{g}=\gamma+C'V^{-1}(y-\alpha) \quad \text{.....(9.1)}$$

where $\hat{g}$ is the predicted breeding value of the parent, and γ is the expected breeding value.

If all entries are from the same population, γ is assumed to be zero. If the entries are from different populations or generations, γ cannot be assumed zero; rather, it is a fixed genetic effect. In our case, all families were not from the same population, but from different populations scattered in different areas and islands, or from two distinctive generations. Therefore, our model should include the fixed genetic effects like "island", "area", and "population". However, very few families in the same trial were from the

same population; rather, in most cases, most families were from different locations (provenances) of 4 major islands. Therefore, the effects of population, area, and island were confounded, and it is impractical to treat them as separate effects. To simplify the model, all families from the same island were treated as families from the same population, and only the fixed island effect was defined in the mixed model.

y is the observed value, and α is the fixed effect including general mean of the trial and other fixed genetic effect like island.

To simplify calculation, family means were used to predict breeding values. Therefore, the single-tree model is then reduced to:

$\overline{Y}_l=\mu+I_k+f_l+fb_l+w_l$

where

μ = fixed general mean,

$\overline{Y}_l$ = the mean of l^{th} family,

I_k = the fixed effect of k^{th} island,

f_l = the random effect of l^{th} family,

fb_l = the random interaction effect of l^{th} family and block,

w_l = the random error of l^{th} family.

The C in White and Hodge's Formula 9.1 is an n x q matrix of covariance between the observed data (n) and the families (q) to be predicted. Since our data were from single-site trials, and only the breeding values of one trait would be predicted, the C matrix was a q x q diagonal matrix. The element on the diagonal was covariance between the family mean and family breeding value. For half-sib families, the covariance between family mean and family breeding value is 2 σ_f, where σ_f is the

variance of family, which is assumed to be the additive genetic effect for open pollinated half-sib families of a diploid species. Koa is primarily a self-incompatible and open-pollinated species (Daehler et al., 1999; Sun, personal comm.), and a diploidized tetraploid (Brewbaker, 1977), thus the assumption holds valid for koa.

V is an n x n variance-covariance matrix of the observed data. Since we assumed all koa families were unrelated, no covariance of the families was counted, and the V matrix was simplified to a diagonal matrix with the variances of family mean on the diagonal of the matrix.

According to the defined model, the variance of family mean was calculated by:

$Var(\bar{y}_l) = \sigma^2_f + \sigma^2_{fb}/n + \sigma^2_w/nb$

where

σ^2_f is the variance component of the family,

σ^2_{fb} is the variance component of the interaction of the family and the block,

$\sigma^2_{w\sigma}$ is the variance of a single tree among the families,

n is the harmonic mean of tree numbers in all blocks,

b is the number of blocks.

The variance components were calculated by "PROC VARCOMP" of SAS® using method=REML.

The fixed effects of α must be known in order to solve the formula 9.1. In our model, the fixed effects included μ (fixed general mean) and I_k (effect of island). With more than two fixed effects, the method of ordinary least squares (OLS) was used to estimate these effects.

The equation for OLS is:

$\hat{\beta} = (X'X)^{-}(X'Y)$

where, $\hat{\beta}$ is the fixed effect, X is the incident matrix of μ and I_k, and Y is the family means (White and Hodge, 1988).

The estimation of the variance among predictions was:

$Var(\hat{g}) = C'V^{-1}C$

The error variance of the predictions was:

$Var(\hat{g} - g) = G - C'V^{-1}C$

where G is known genetic variance and covariance. In our case, $G = 4*\sigma^2_f$.

The correlation between true and predicted breeding values was:

$Corr(\hat{g},g) = \{Var(\hat{g})/Var(g)\}^{1/2}$

All three parameters were used to assess the precision of predictions (White and Hodge, 1989), and only the correlation ($Corr(\hat{g},g)$) between true and predicted breeding values will be discussed due to its explicitness.

Results

Family means of DBH and the numbers of surviving trees of the families at age 4 are summarized in Table 9.1 (only data of replicated families were included in the table). All families showed great differences in DBH at age 4. ANOVA of 4 trials using single-tree data are presented in Table 9.2. The differences among islands were significant in three trials (SET 91-1, 95-1, and 97-1), and the differences among families within islands were significant in two trials (SET 95-1 and 97- 1), but not in SET 91-1. In SET 98-1, both the difference between the selected populations and the control, and the difference among families within the selected populations and the control were not significant.

Table 9.1. Family means of DBH and numbers of surviving trees in the families.

A. SET 91-1

Family	Mean No. of surviving trees	Family mean (cm)	Family	Mean No. of surviving trees	Family mean (cm)
1MC	5.0	10.69	2NU2C	5.0	12.95
1N1-3	5.0	11.06	2PH1-1	5.0	14.23
1N1-4	5.0	8.57	2PH2-1	4.8	9.13
1N1-5	4.0	10.74	2PK3-1	4.4	14.65
1PU1-2	4.6	8.42	5K1-1	5.0	11.15
1Sl3-1	3.9	8.02	5OL1-2	4.4	6.20
1SL3-5	5.0	8.21	6-0191	2.4	5.80
2HA1C	4.7	10.86	6-1288b	4.0	7.37
2KU1-1	4.8	11.04	6-1288c	3.4	7.22
2KU2-1	5.5	10.51	6-1288d	4.7	6.67
2ML1-1	5.0	11.00	6KA1-1	3.6	9.34

B. SET 95-1

# of surviving trees				# of surviving trees			
Family	Rep1	Rep2	Family mean (cm)	Family	Rep1	Rep2	Family mean (cm)
1PP1C	7	9	5.74	2W4-3	10	10	7.96
1PT1C	8	10	9.25	2WT1-4A	8	7	6.16
1WM1C	5	3	8.29	5CP1-2	8	10	7.68
2AA1-5	5	4	4.47	5CP1-3	7	4	6.32
2HL1-1	9	8	5.89	5CP1-5	10	9	7.06
2KH2-1	9	8	8.14	5GUL1C	7	9	7.66
2KU1C3	10	9	7.29	5KH1C	10	10	6.52
2KW1-2	10	8	8.45	5KO1-1	4	3	5.21
2KW1-4	10	10	9.42	5KP1C	6	8	9.95
2KW2-1	9	10	8.54	5MA1-2	9	7	7.61
2KW2-2	9	10	8.18	5SM1-2	10	10	8.38
2KW3-1	9	9	8.59	6HAM1C	5	9	4.97
2ML2-1	9	7	6.01	6HK1C	2	5	2.61
2NU2-1	10	10	7.78	6HK2C	2	6	5.73
2PH2-2	10	5	9.54	6KH1C	9	8	5.40
2PH2-3	7	10	7.31	6KH2C	6	4	4.61
2PK1-2	10	10	8.34	6KM1C	5	6	6.69
2PK1-3	3	10	7.18	6KP1C	7	4	5.99
2PK2-1	8	10	6.76	6KU1C	3	2	2.69
2PK2-1B	6	8	4.65	6ML1C	7	9	3.89
2PK2-2	5	9	8.07	6ML2C	4	7	3.28
2PK3-1	10	7	8.64	6MLR5-1	6	4	4.89
2PK3-1b	10	10	7.31	6OF1C	8	5	8.58
2PO1-2	7	6	4.27	6PUU1C	9	9	6.42
2W2-2	10	9	8.44	6SAD1C	5	5	6.37
2W3-4	9	10	8.53	6WV1-1	9	3	6.58
2W4-2	6	7	4.39	6WV1-2	7	6	7.73

Table 9.1. Continued. Family means of DBH and numbers of surviving trees in the families.

C. SET 97-1

# of surviving trees				# of surviving trees			
Family	Rep1	Rep2	Family mean (cm)	Family	Rep1	Rep2	Family mean (cm)
1MA1-1	4	8	10.40	6HO3-4	4	3	9.91
1MA1-2(97)	8	3	10.66	6HO4-1	8	4	5.60
1MC1-1	9	5	6.29	6KE1-1	3	4	3.89
1MN1-5	4	6	7.49	6KE1-2	7	2	6.42
1MN1-6	4	5	7.34	6KE1-3	7	6	4.25
1NA1-1	4	4	9.90	6KE1-4	6	3	8.14
1WL1-2	2	4	5.40	6KEA1-10	2	3	4.38
2HA1C(91s)	4	5	11.54	6KEA1-11	4	4	4.93
2KP2-1(94)	1	2	9.45	6KEA1-12	1	6	3.58
2KU2-1(91s)	3	2	7.73	6KEA1-14	8	9	6.99
2KW2-2(95)	1	2	4.95	6KEA1-2	3	5	3.45
2MA2-1(94)	8	6	7.09	6KEA1-3	1	1	5.90
2PH2-2(94)	1	2	8.05	6KEA1-4	5	8	4.11
2PH2-3(95)	2	3	6.98	6KEA1-5	4	5	4.97
6HO1-1	5	4	5.59	6KEA1-6	4	4	5.08
6HO1-10	1	6	8.01	6KEA1-8	3	5	4.77
6HO1-11	6	5	7.07	6LA1-1	4	4	9.04
6HO1-2	8	7	5.34	6LA1-4	2	7	10.79
6HO1-3	9	7	4.29	6UM1-1	6	6	7.83
6HO1-4	6	4	4.74	6UM2-2	4	8	6.69
6HO1-5	6	5	4.15	6UM2-3	5	5	6.00
6HO1-6	5	6	7.24	6UM2-4	8	8	5.38
6HO1-9	3	4	4.40	6UM2-5	2	5	4.72
6HO2-1	9	7	8.17	6UM3-1	6	8	6.56
6HO3-2	7	9	5.55	6UM3-2	4	9	6.67
6HO3-3	5	3	4.26	6UM4-1	4	3	4.43

D. SET 98-1

# of surviving trees				# of surviving trees			
Family	Rep1	Rep2	Family mean (cm)	Family	Rep1	Rep2	Family mean (cm)
1N1-3-91-202-10	2	4	10.42	2ML1--92-204-11	2	9	10.08
1N2-1-93-302-12	3	5	12.15	2NU2c-91-130-6	3	5	12.36
1N2-5-91-154-5	3	4	8.57	2NU2c-91-131-1	4	5	5.31
1NU2-1-93-302-7	4	6	5.53	2NU2c-91-131-3	8	6	8.67
2HA1c-91-222-2	6	6	6.31	2NU2c-91-228-6	6	1	11.24
2ML1-91-212-9	3	4	10.24	2PH2-92-303-5	3	5	7.98
2ML1-92-107-11	5	6	10.82	6KA1-1-91-206-3	1	6	13.02
				Composite, bulked '91 trial	8	8	10.18

Table 9.2. Analysis of variance of 4 progeny trials using single-tree data.

A. SET 91-1

Source	DF	SS	MS	F Value	Pr > F
Rep	1	15.08	15.08	0.82	0.3768
Island	3	845.41	281.8	15.24	<.0001
Family (F) in islands	38	908.78	23.9	1.29	0.2687
Rep*F	21	423.22	20.15	1.27	0.1880
Error	437	6919.97	15.83		

B. SET 95-1

Source	DF	SS	MS	F Value	Pr > F
Rep	1	16.65	16.65	1.39	0.2437
Island	3	462.42	154.14	12.86	<.0001
Family (F) in islands	50	1562.86	31.26	2.61	0.0004
F*Rep	53	635.09	11.98	1.44	0.0238
Error	706	5856.26	8.29		

C. SET 97-1

Source	DF	SS	MS	F Value	Pr > F
Rep	1	10.38	10.38	0.98	0.3273
Island	3	229.11	76.37	7.19	0.0004
Family (F) in islands	71	1967.03	27.70	2.61	0.0002
F*Rep	53	562.88	10.62	1.07	0.3527
Error	460	4573.57	9.94		

D. SET 98-1

Source	DF	SS	MS	F Value	Pr > F
Rep	1	13.49	13.49	0.55	0.4690
Generation	1	67.54	67.54	2.73	0.1148
Family (F) in gen.	24	796.72	33.20	1.34	0.2582
F*Rep	19	469.74	24.72	1.11	0.3481
Error	110	2442.83	22.20		

Significant effects of islands indicated that families from certain islands were generally superior to those from other islands, to be discussed later.

Variance components on random model are given in Table 9.3 and were fairly similar, indicating the four trials had similar precisions. All four trials were located in the same location, and the site preparation and the management of the trials were similar and carefully done.

Table 9.3. Variance components of 4 progeny trials.

	Variance component		
SET	σ^2_f	σ^2_{fb}	σ^2_e
91-1	1.12	0.62	12.93
95-1	1.45	0.41	8.36
97-1	1.93	0.42	10.23
98-1	1.73	0.00	22.13

Using ordinary least squares method, general mean and island effects of each trial are given in Table 9.4. The estimated general means among 4 trials were slightly different. If all the families in one trial were random samples of the species, such discrepancy could be regarded as the fixed effect of the trial. Generally, families from Oahu and Kauai were superior to those from Maui and Hawaii. The former families had positive island effects and the latter ones had negative island effects.

Table 9.4. Fixed island effect (cm) of 4 progeny trials at the age of 4 years.

	SET 91-1	SET 95-1	SET 97-1	SET 98-1
General mean	9.46	6.94	7.06	6.86(control)
Oahu	0.44	0.82	0.85	
Kauai	1.94	0.38	0.49	
Maui	-0.22	0.43	-0.79	
Hawaii	-2.17	-1.62	-0.54	

Predicted breeding values of DBH for SET 91-1 (Table 9.5) at the age of 4 years ranged from –2.99 cm of family 6-0191 to 4.62 cm of family 2PK3-1. Families with the highest predicted breeding values were 2PK3-1, 2PH1-1, and 2NU2c.

Table 9.5. Predicted breeding values of DBH of SET 91-1 at the age of 4 years.

Family	Family mean (cm)	Breeding value (cm)	V($\hat{g}$)	V($\hat{g}$ -g)	Corr($\hat{g}$,g)
6-0191	5.80	-2.99	2.65	1.83	0.77
6-1288d	6.67	-2.89	2.38	2.10	0.73
5OL1-2	6.20	-2.73	1.98	2.50	0.66
6-1288c	7.22	-2.24	1.74	2.74	0.62
6-1288b	7.37	-2.08	2.91	1.57	0.81
1Sl3-1	8.02	-1.53	2.38	2.10	0.73
1PU1-2	8.42	-1.48	1.98	2.50	0.66
1SL3-5	8.21	-1.05	2.59	1.89	0.76
1N1-4	8.57	-0.73	3.16	1.32	0.84
2PH2-1	9.13	-0.43	2.07	2.41	0.68
6KA1-1	9.34	-0.08	3.20	1.28	0.85
1N1-5	10.74	1.09	2.38	2.10	0.73
2KU2-1	10.51	1.12	1.98	2.50	0.66
2HA1C	10.86	1.31	2.34	2.14	0.72
1MC	10.69	1.37	1.85	2.63	0.64
2ML1-1	11.00	1.37	2.38	2.10	0.73
2KU1-1	11.04	1.44	1.85	2.63	0.64
1N1-3	11.06	1.67	1.23	3.25	0.52
5K1-1	11.15	1.82	2.41	2.07	0.73
2NU2C	12.95	3.59	2.23	2.25	0.71
2PH1-1	14.23	4.44	2.59	1.89	0.76
2PK3-1	14.65	4.62	2.29	2.19	0.72

Predicted breeding values for SET95-1 (Table 9.6) ranged from –4.19 cm of 6HK1C to 3.96 cm of 5KP1C. Families with the highest predicted breeding values were 1PT1C, 2PH2-2, 2KW1-4, and 5KP1C.

Table 9.6. Predicted breeding values of DBH of SET 95-1 at the age of 4 years.

Family	Family mean (cm)	Breeding value (cm)	$V(\hat{g})$	$V(\hat{g}-g)$	$Corr(\hat{g},g)$	Family	Family mean (cm)	Breeding value (cm)	$V(\hat{g})$	$V(\hat{g}-g)$	$Corr(\hat{g},g)$
6HK1C	2.61	-4.19	4.14	1.66	0.84	2PK1-3	7.18	0.22	4.41	1.39	0.87
6ML2C	3.28	-4.14	4.28	1.52	0.86	2KU1C3	7.29	0.34	4.07	1.73	0.84
6KU1C	2.69	-3.89	3.15	2.65	0.74	2PH2-3	7.31	0.37	4.28	1.52	0.86
2PO1-2	4.27	-3.72	3.39	2.41	0.76	2PK3-1b	7.31	0.37	3.58	2.22	0.79
6ML1C	3.89	-3.66	4.22	1.58	0.85	5MA1-2	7.61	0.78	4.35	1.45	0.87
2W4-2	4.39	-3.56	4.22	1.58	0.85	5GUL1C	7.66	0.85	4.14	1.66	0.84
2PK2-1B	4.65	-3.27	4.35	1.45	0.87	5CP1-2	7.68	0.89	4.41	1.39	0.87
2AA1-5	4.47	-2.95	4.28	1.52	0.86	2NU2-1	7.78	1.08	3.02	2.78	0.72
6KH2C	4.61	-2.47	4.41	1.39	0.87	2W4-3	7.96	1.36	3.97	1.83	0.83
6MLR5-1	4.89	-2.14	4.35	1.45	0.87	2PK2-2	8.07	1.38	4.14	1.66	0.84
6HAM1C	4.97	-2.09	4.35	1.45	0.87	1WM1C	8.29	1.39	4.41	1.39	0.87
1PP1C	5.74	-2.06	4.29	1.51	0.86	2KH2-1	8.14	1.58	3.89	1.91	0.82
5KO1-1	5.21	-1.82	4.14	1.66	0.84	2KW2-2	8.18	1.67	2.75	3.05	0.69
2HL1-1	5.89	-1.70	4.41	1.39	0.87	6WV1-2	7.73	1.72	2.82	2.98	0.70
6KH1C	5.40	-1.51	3.93	1.87	0.82	2PK1-2	8.34	1.93	4.22	1.58	0.85
2MI2-1	6.01	-1.49	4.19	1.61	0.85	5SM1-2	8.38	1.97	3.50	2.30	0.78
2WT1-4A	6.16	-1.25	4.41	1.39	0.87	2KW1-2	8.45	2.05	3.67	2.13	0.80
6HK2C	5.73	-1.22	3.44	2.36	0.77	2W2-2	8.44	2.06	3.58	2.22	0.79
5CP1-3	6.32	-0.87	4.28	1.52	0.86	2W3-4	8.53	2.20	2.50	3.30	0.66
5KH1C	6.52	-0.87	3.97	1.83	0.83	2KW2-1	8.54	2.20	4.14	1.66	0.84
6KP1C	5.99	-0.79	3.89	1.91	0.82	2KW3-1	8.59	2.26	3.58	2.22	0.79
2PK2-1	6.76	-0.45	4.19	1.61	0.85	2PK3-1	8.64	2.29	3.50	2.30	0.78
6SAD1C	6.37	-0.33	4.41	1.39	0.87	6OF1C	8.58	2.69	3.83	1.97	0.81
6WV1-1	6.58	-0.18	3.89	1.91	0.82	1PT1C	9.25	3.02	4.29	1.51	0.86
5CP1-5	7.06	-0.03	4.35	1.45	0.87	2PH2-2	9.54	3.40	3.55	2.25	0.78
6PUU1C	6.42	0.01	4.35	1.45	0.87	2KW1-4	9.42	3.57	3.31	2.49	0.76
6KM1C	6.69	0.11	3.89	1.91	0.82	5KP1C	9.95	3.96	4.03	1.77	0.83

Predicted breeding values for SET 97-1 (Table 9.7) ranged from –4.02 cm of family 6KEA1-2 to 5.34 cm of family 2HA1C(91s). Families with the highest predicted breeding values were 6LA1-4, 1MA1-1, 1MA1-2(97), and 2HA1C(91s).

Table 9.7. Predicted breeding values of DBH of SET 97-1 at the age of 4 years.

Family	Family mean (cm)	Breeding value (cm)	$V(\hat{g})$	$V(\hat{g}-g)$	$Corr(\hat{g},g)$	Family	Family mean (cm)	Breeding value (cm)	$V(\hat{g})$	$V(\hat{g}-g)$	$Corr(\hat{g},g)$
6KEA1-2	3.45	-4.02	4.37	2.45	0.83	6UM2-2	6.69	-0.84	5.02	2.38	0.83
6KEA1-4	4.11	-3.83	5.27	2.06	0.86	1MC1-1	6.29	-0.84	5.34	3.70	0.72
6HO1-3	4.29	-3.80	5.66	2.37	0.83	6KE1-2	6.42	-0.65	4.02	2.28	0.84
6KE1-3	4.25	-3.68	5.35	2.66	0.81	6UM3-1	6.56	-0.48	5.44	2.63	0.81
6HO1-5	4.15	-3.65	5.06	3.52	0.74	6UM3-2	6.67	-0.34	5.09	2.28	0.84
6KE1-1	3.89	-3.41	4.20	3.35	0.75	2MA2-1(94)	7.09	-0.15	5.44	4.20	0.67
6HO3-3	4.26	-3.11	4.37	3.52	0.74	2PH2-3(95)	6.98	0.06	3.52	2.66	0.81
6HO1-9	4.40	-2.84	4.20	3.52	0.74	6HO1-11	7.07	0.06	5.06	3.03	0.78
6UM4-1	4.43	-2.81	4.20	2.89	0.79	1MN1-6	7.34	0.16	4.69	1.95	0.86
6HO1-4	4.74	-2.77	4.83	4.83	0.61	6KEA1-14	6.99	0.17	5.77	2.89	0.79
6KEA1-12	3.58	-2.74	2.89	3.35	0.75	1MN1-5	7.49	0.32	4.83	2.66	0.81
6KEA1-8	4.77	-2.53	4.37	4.2	0.67	6HO1-6	7.24	0.40	5.06	4.83	0.61
6KEA1-10	4.38	-2.49	3.52	3.03	0.78	6HO1-10	8.01	0.58	2.89	4.20	0.67
6KEA1-5	4.97	-2.43	4.69	3.22	0.76	2KU2-1(91s)	7.73	0.66	3.52	5.27	0.56
6KEA1-11	4.93	-2.39	4.50	3.87	0.71	2PH2-2(94)	8.05	0.81	2.45	2.50	0.82
6UM2-5	4.72	-2.34	3.85	2.14	0.85	6UM1-1	7.83	1.23	5.22	3.22	0.76
6HO1-2	5.34	-2.25	5.58	3.22	0.76	6KE1-4	8.14	1.34	4.50	5.27	0.56
6KEA1-6	5.08	-2.22	4.50	2.04	0.86	2KP2-1(94)	9.45	1.70	2.45	2.06	0.86
6UM2-4	5.38	-2.22	5.68	2.06	0.86	6HO2-1	8.17	1.88	5.66	3.22	0.76
6HO3-2	5.55	-1.96	5.66	2.70	0.81	6LA1-1	9.04	2.40	4.50	3.52	0.74
6HO4-1	5.60	-1.73	5.02	3.03	0.78	6HO3-4	9.91	3.16	4.20	3.22	0.76
6HO1-1	5.59	-1.67	4.69	4.00	0.69	1NA1-1	9.90	3.17	4.50	3.70	0.72
1WL1-2	5.40	-1.57	3.72	2.81	0.80	6LA1-4	10.79	3.90	4.02	2.70	0.81
6UM2-3	6.00	-1.20	4.91	5.27	0.56	1MA1-1	10.4	4.09	5.02	3.06	0.78
2KW2-2(95)	4.95	-1.16	2.45	5.72	0.51	1MA1-2(97)	10.66	4.17	4.66	3.03	0.78
6KEA1-3	5.90	-0.86	2.00	2.70	0.81	2HA1C(91s)	11.54	5.34	4.69	2.45	0.83

Predicted breeding values for SET 98-1 (Table 9.8) ranged from –1.10 cm of family 1NU2-1-93-302-7 to 3.90 cm of family 1N2-1-93-302-12. Families with the highest predicted breeding values were 6KA1-1-91-206-3, 2NU2c-91-130-6, 2ML1-92-107-11, and 1N2-1-93-302-12.

Table 9.8. Predicted breeding values of DBH of SET 98-1 at the age of 4 years.

Family	Family mean (cm)	Breeding value (cm)	V(ĝ)	V(ĝ -g)	Corr(ĝ,g)
1NU2-1-93-302-7	5.53	-1.10	2.84	4.08	0.64
2NU2c-91-131-1	5.31	-1.09	2.41	4.51	0.59
2HA1c-91-222-2	6.31	-0.52	3.19	3.73	0.68
2PH2-92-303-5	7.98	0.71	2.21	4.71	0.57
1N2-5-91-154-5	8.57	1.00	3.21	3.71	0.68
2NU2c-91-131-3	8.67	1.67	2.04	4.88	0.54
2NU2c-91-228-6	11.24	1.75	2.14	4.78	0.56
2ML1--92-204-11	10.08	1.98	2.21	4.71	0.57
1N1-3-91-202-10	10.42	2.09	2.41	4.51	0.59
Composite, bulked '91 trial	10.18	2.11	2.04	4.88	0.54
2ML1-91-212-9	10.24	2.35	1.38	5.54	0.45
6KA1-1-91-206-3	13.02	2.60	2.41	4.51	0.59
2NU2c-91-130-6	12.36	2.62	1.65	5.27	0.49
2ML1-92-107-11	10.82	2.76	1.46	5.46	0.46
1N2-1-93-302-12	12.15	3.90	2.56	4.36	0.61

Most of families with high predicted breeding values were from Oahu, Kauai, and Maui. Only few families with high predicted breeding values were from Hawaii Island. The results indicated that families from islands other than Hawaii had greater potential in DBH growth than those from Hawaii Island.

The rankings of families based on predicted breeding values were generally identical to those based on family means. Some exceptions existed. Some families had different ranks based on predicted breeding values and family means, evidently caused by different numbers of surviving trees in the families. Families with higher survival rates

had smaller error variances of family means (C in Formula 9.1) and higher predicted breeding values if the family means were the same.

The precision of predicting breeding values was measured by three parameters, Var($\hat{g}$)--the variance among the predictions; Var($\hat{g}$ -g)--the error variance of the predictions; and Corr($\hat{g}$, g)--the correlation of predicted breeding value and true breeding value. Since Var($\hat{g}$) and Var($\hat{g}$ -g) were values of variance, it is difficult to use them to compare the prediction precision among different trials and within a trial. It is more convenient to use Corr($\hat{g}$, g) as a parameter to measure the precision. Larger Corr($\hat{g}$, g) means that the correlation between predicted breeding value and true breeding value is higher, thus the prediction is more precise. The prediction precision of SET 95-1 and SET 97-1 was high, with most Corr($\hat{g}$, g) of more than 0.8. While the precision of SET 91-1 and 98-1 was relatively low with most Corr($\hat{g}$, g) of around 0.7 and 0.6. Lower Corr($\hat{g}$, g) was caused by the higher experimental error of the trial. Different prediction precisions also occurred among families within the predicted trials, resulted from different survival rates of the families.

Predicated breeding values of the top 10% families across all four trials are listed in Table 9. 9. All four trials had families with high predicted breeding values. Most of selected families were from Kauai (7 out of 15) and Oahu (5 out of 15). Only 3 out of 15 selected families came from Hawaii and Maui. The results were similar to those of Sun's (1996) work based on phenotypic analysis of early growth of koa.

Table 9.9. Families with the highest predicted breeding values.

Family	Family mean (cm)	Predicted breeding value (cm)	SET
2ML1-92-107-11	10.82	2.76	98-1
1PT1C	9.25	3.02	95-1
6HO3-4	9.91	3.16	97-1
1NA1-1	9.90	3.17	97-1
2PH2-2	9.54	3.40	95-1
2KW1-4	9.42	3.57	95-1
2NU2C	12.95	3.59	91-1
6LA1-4	10.79	3.90	97-1
1N2-1-93-302-12	12.15	3.90	98-1
5KP1C	9.95	3.96	95-1
1MA1-1	10.40	4.09	97-1
1MA1-2(97)	10.66	4.17	97-1
2PH1-1	14.23	4.44	91-1
2PK3-1	14.65	4.62	91-1
2HA1C(91s)	11.54	5.34	97-1

Using predicated breeding values, predicted genetic gain was calculated for selected families. If the top 10% of families based on predicted breeding values are selected for all 4 trials, the calculated average predicted genetic gain is 3.8 cm. This means that the average DBH of half-sib seedlings from those families will be 1.9 cm larger in DBH than overall mean DBH of the species at the same age (since half-sib seedlings only inherit half breeding values of mother trees). If those families could be cloned or used to produce seeds from a clonal seed orchard, the genetic gain of those clones or seeds will be 3.8 cm.

Discussion

The four trials were at a single site planted in different years with different sets of half-sib families. Methods of family ranking such as standard scores and least squares are thus inappropriate to compare families across such trials. Those methods are more appropriate for multiple-site trials represented by identical families (Hatcher, et al., 1981;

Cotterill et al., 1983). It can also be inappropriate to rank families across different trials based on family means, since different experimental errors among the trials, plus fixed effects of the trials make direct comparison less meaningful. Although generally our four sets of trials had similar variance structures (Table 9.3), small differences of experimental error among the trials should still be considered in a critical ranking. On the other hand, family ranking based on predicted breeding values made the comparison of families in different trials more comparable, since such ranking was based on "pure", to some extent, genetic effects of families, and errors in estimating such genetic effects were minimized (White and Hodge, 1988).

Some cautions must be taken when interpreting the results. Because all families occurred only in one trial at one site, and common controls were not always planted, genetic effect of the family was confounded with interaction between family and trial, as well as interaction between family and site, which might exaggerate genetic effects of families (Ma, 1999). Thus the prediction of breeding values of the families might also be exaggerated. In the future, it would be valuable to test families in multiple sites to evaluate the interaction of family by environment (Ma, 1999).

A great advantage of BLP method is that the data of different traits at different ages and sites can be incorporated into the prediction of breeding values of those traits (White and Hodge, 1988). In present study, we were only able to use DBH data at age four because of data availability. We believe that if data of more traits are available, the prediction will be more precise since the correlation between different traits will be counted into the prediction. In the future, data of more traits such as height, straightness

of bole, and resistance to disease should be gathered to better evaluate the performance of families.

Twelve out of 15 selected families (top 10%) based on predicted breeding values of DBH were from Kauai and Oahu, and only three families were from Hawaii and Maui. Family composition of all four trials was 12.5, 33.6, 7.8, and 46.1% of families from Oahu, Kauai, Maui, and Hawaii respectively. The number of selected families from Kauai and Oahu was disproportionately high compared to their portion in total families in the trials (46% of total families vs. 80% of selected families). It clearly indicates that koa germplasm of Kauai and Oahu was superior to that of Hawaii in terms of early DBH. Sun (1996) drew a similar conclusion based on phenotypic analysis of early growth of koa at Hamakua and Maunawili. Dudley (2003) also found that the best families of his trials at Maunawili were from some areas of Oahu and Kauai. New findings were contradictory to such an old concept that families with long, branch-free log were from Hawaii only (Skolmen, 1990). More attention should be paid to the germplasm of Kauai, Oahu, and Maui as suggested by Sun (1996).

Predicted genetic gain of top 10% families was high (3.8 cm of DBH at age 4). This could translate into 54% increase above the average performance of the species, if the overall DBH of the species is 7 cm at age 4 (Table 9.3). In real breeding programs, actual genetic gain will differ from predicted one depending on experimental error and selection methods used. If backward selection is conducted, which means to trace back to mother trees of the selected families, either by directly using clones of the mother trees or by establishing a seed orchard based on the clones of the mother trees, the genetic gain of those clones or seeds of the clones will be maximized to 3.8 cm under ideal conditions.

If half-sib seeds of those selected families are used, since those half-sib seeds only inherit half genetic worth of the mother trees, the predicted genetic gain of those half-sib seeds will be only half of that of the mother trees, e.g. 1.9 cm at age 4. Selection can also be made on individual trees within selected families, and the genetic gain of such selection will depend on the heritibility and selection intensity of individual trees.

Conclusions

Great variations in DBH at the age of 4 years were found among koa families from 4 islands in 4 trials. Similar variance structures of the 4 trials indicated that they had similar precision. Breeding values predicted by BLP made families from different trials more comparable, thus family ranking across different trials based on predicted breeding values was more accurate than ranking based on family means. Generally, koa families had high predicted breeding values and prediction precision at the age of 4 years. Fifteen families were selected based on predicted breeding values. Genetic gains were estimated 3.8 cm and 1.9 cm under backward selection and forward selection models.

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